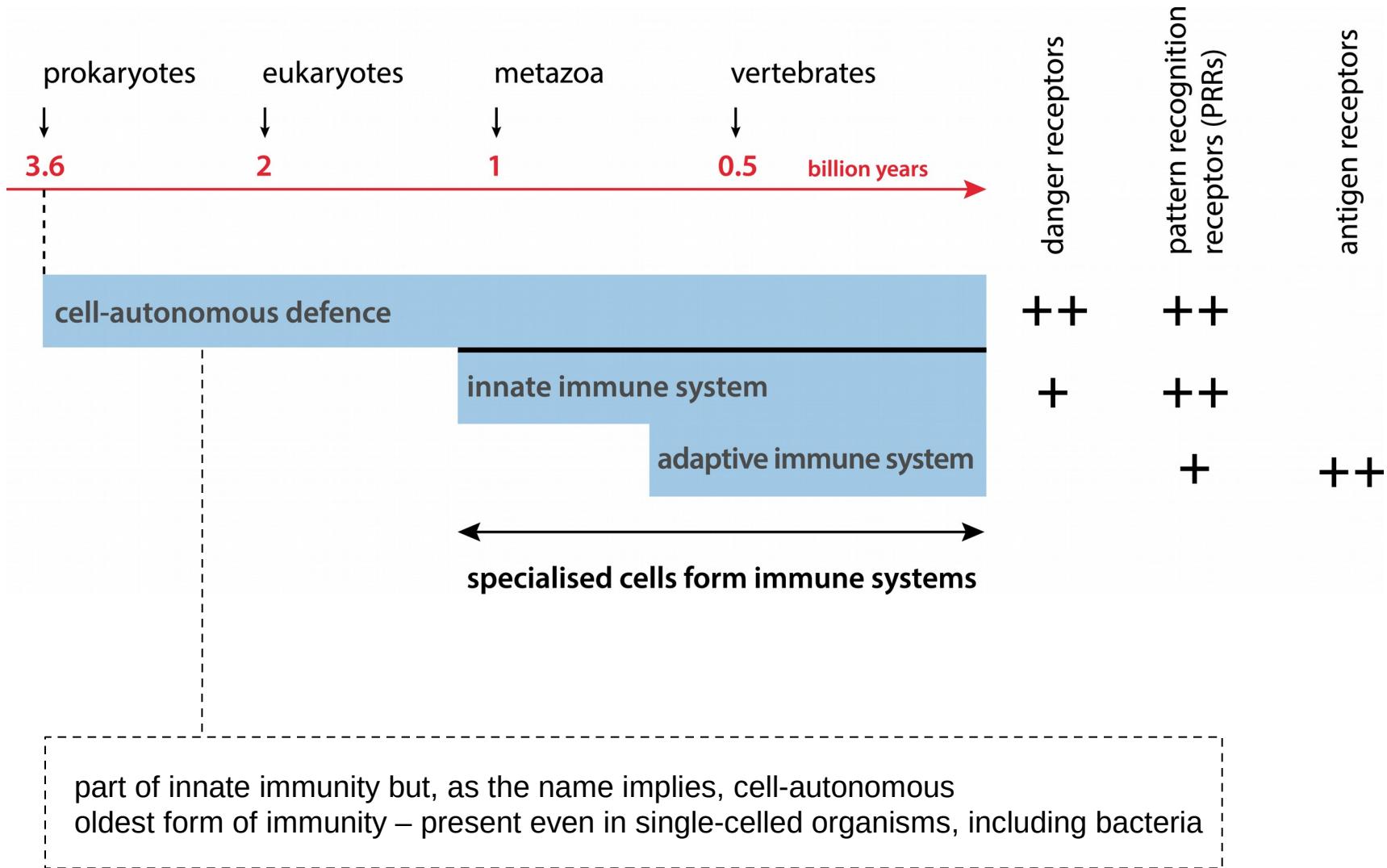
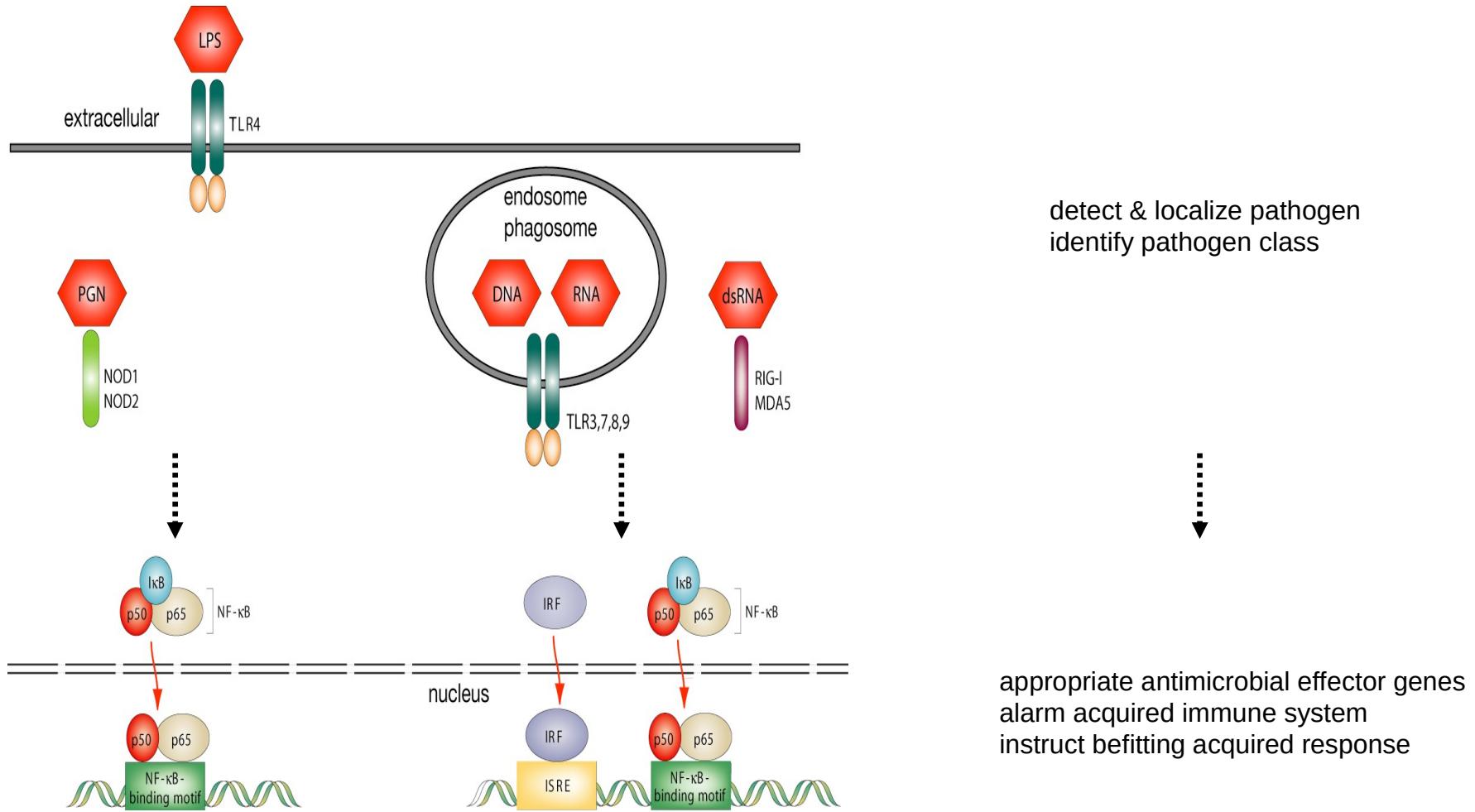


# Cell-autonomous defence and canonical immunity



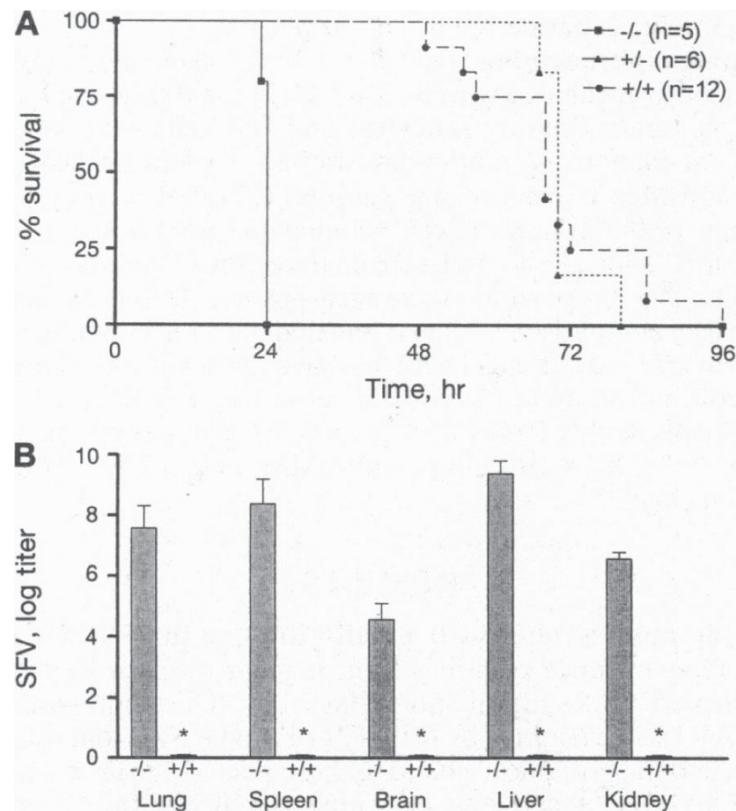
# A wide range of pattern recognition receptors protects us



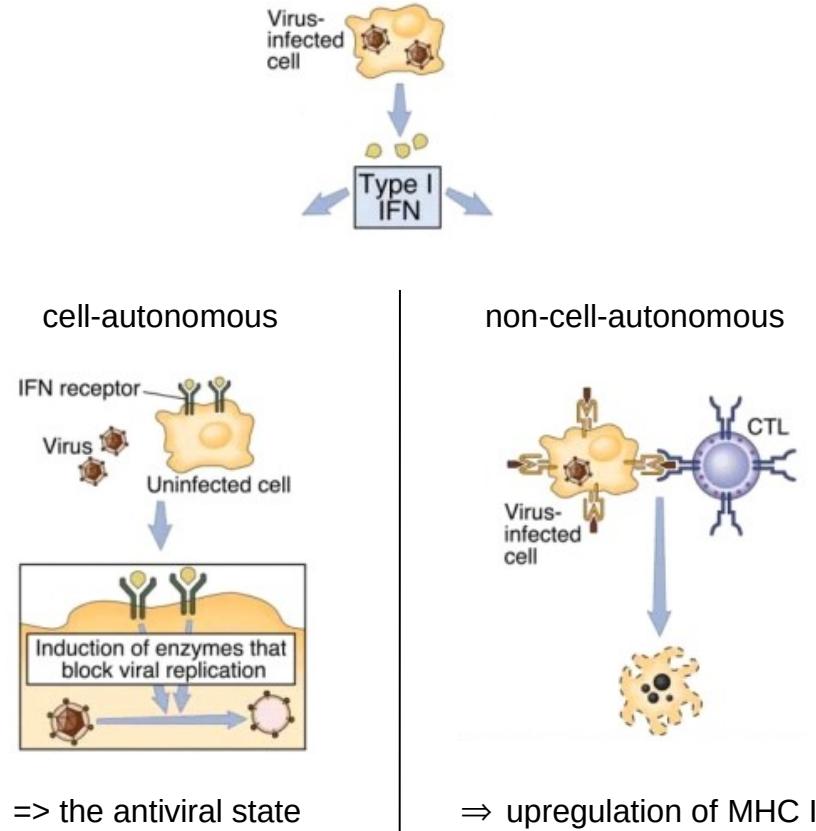
# Innate protection against viruses by type 1 interferon

Semliki Forest virus into

interferon receptor-deficient mice (IFNAR1<sup>-/-</sup>)



Hwang, PNAS 1995



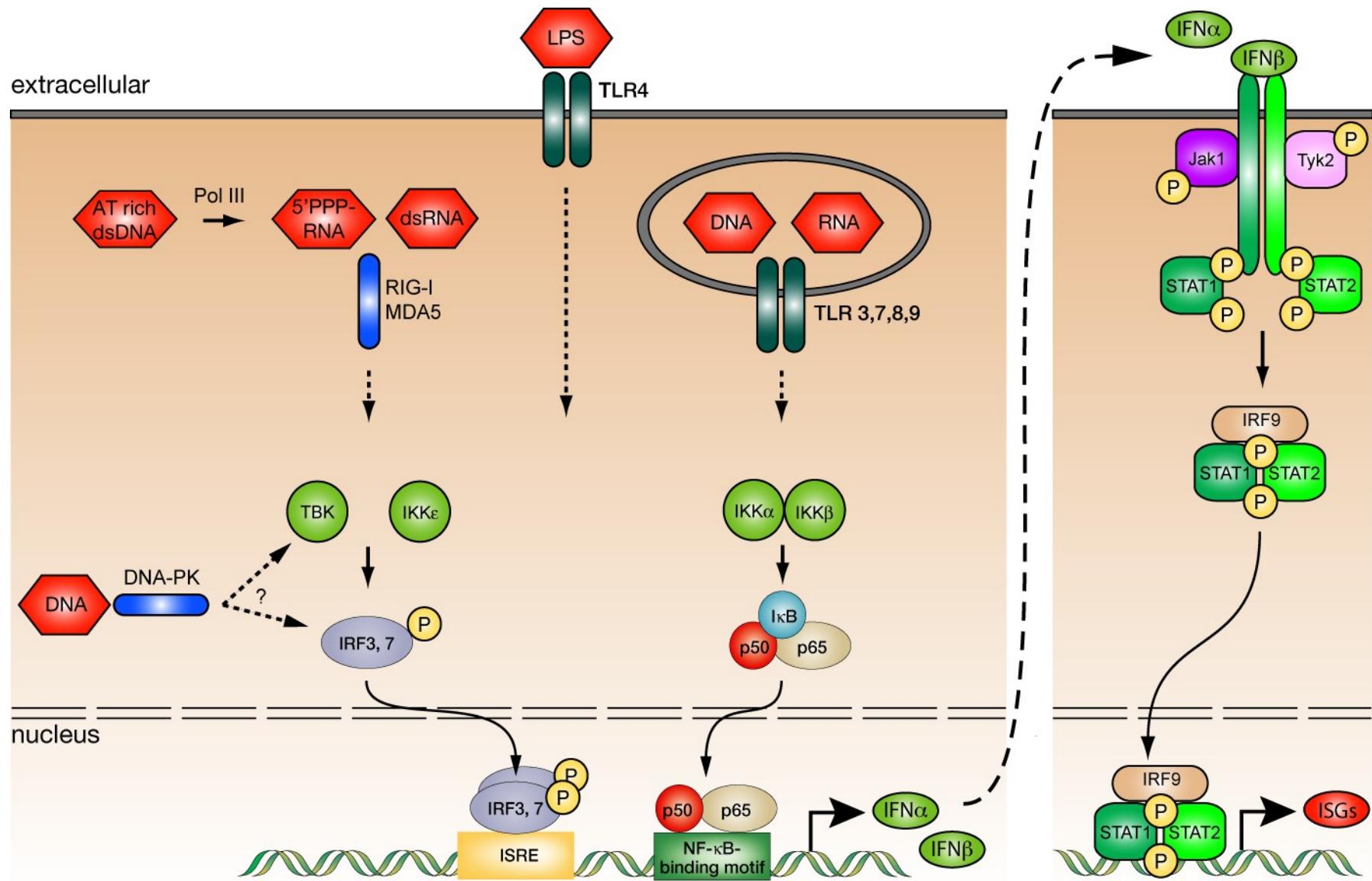
# Interferons

typical cytokines: small (~15kDa), dimeric secreted proteins

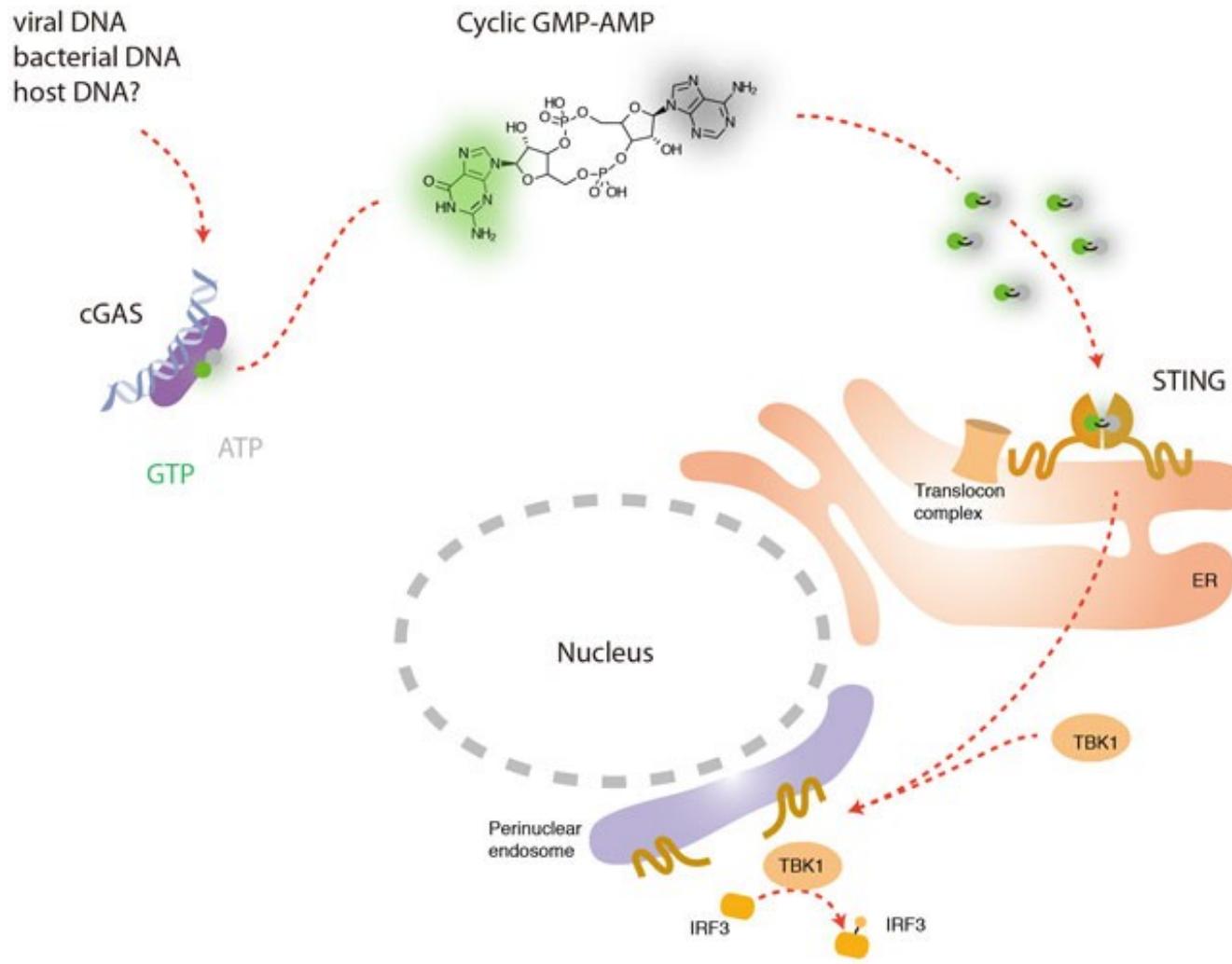
Table 1 | Members of the IFN family of cytokines

Feature	Type I IFNs	Type II IFN	Type III IFNs
Subtypes	IFN- $\alpha$ (13 subtypes) IFN- $\beta$ IFN- $\omega$ IFN- $\epsilon$ IFN- $\kappa$	IFN- $\gamma$	IFN- $\lambda$ -1(also known as IL-29) IFN- $\lambda$ -2 (also known as IL-28A) IFN- $\lambda$ -3 (also known as IL-28B)
Chromosome localization	9p22	12q14	19q13
Produced by	All nucleated cells	NK cells NKT cells T cells	All nucleated cells
Receptor subunits	IFNAR1 IFNAR2	IFNGR1 IFNGR2	IFNLR1 IL-10R2
Receptor expression	All nucleated cells	All nucleated cells	Epithelial cells pDCs
Signaling molecules	JAK1 and TYK2	JAK1 and JAK2	JAK1 and TYK2
Transcription factors*	STAT1/STAT2/IRF9 STAT1/STAT1	STAT1/STAT1	STAT1/STAT2/IRF9 STAT1/STAT1

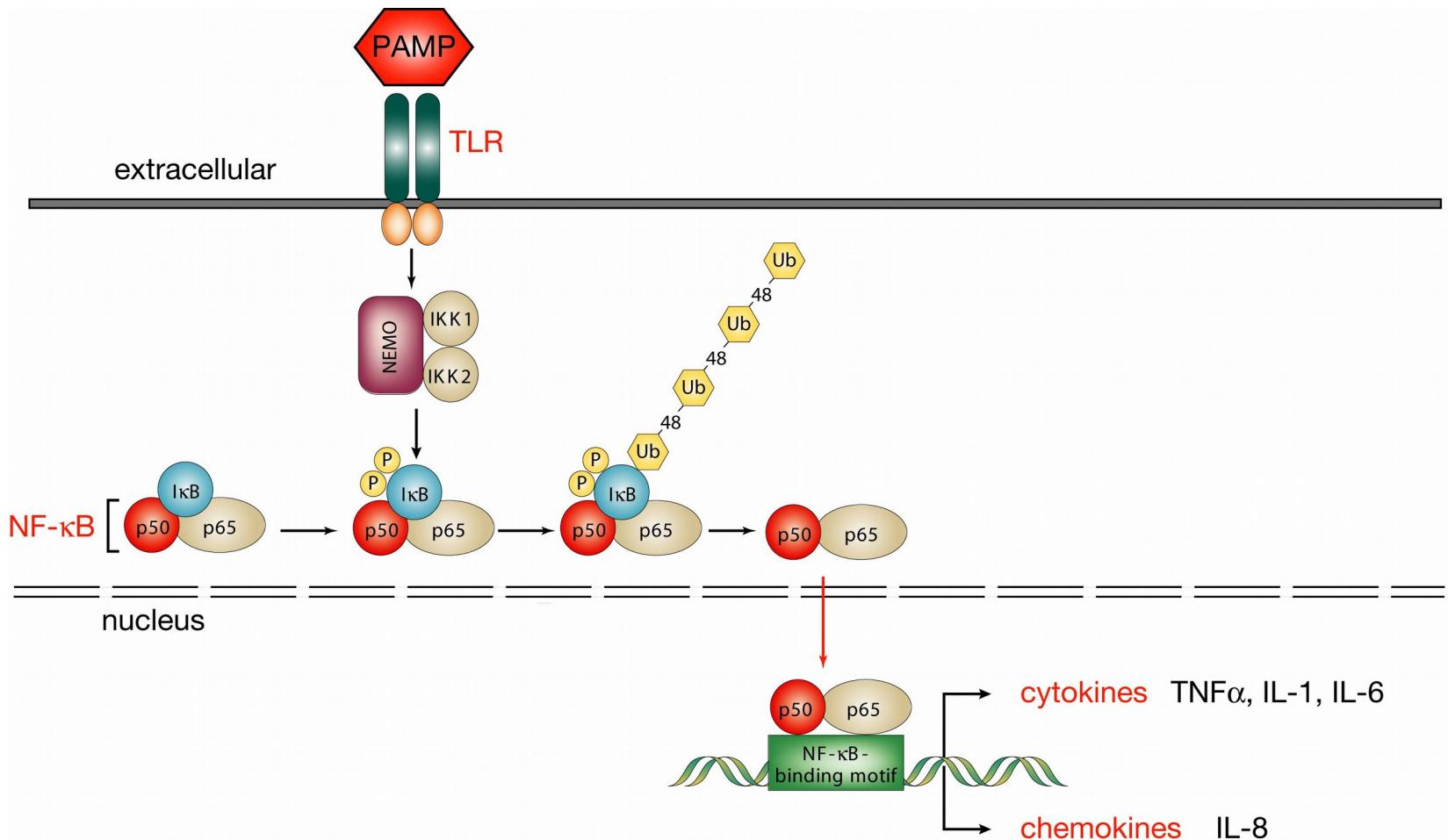
## How cells produce IFN $\alpha$ / $\beta$ and how IFN signals



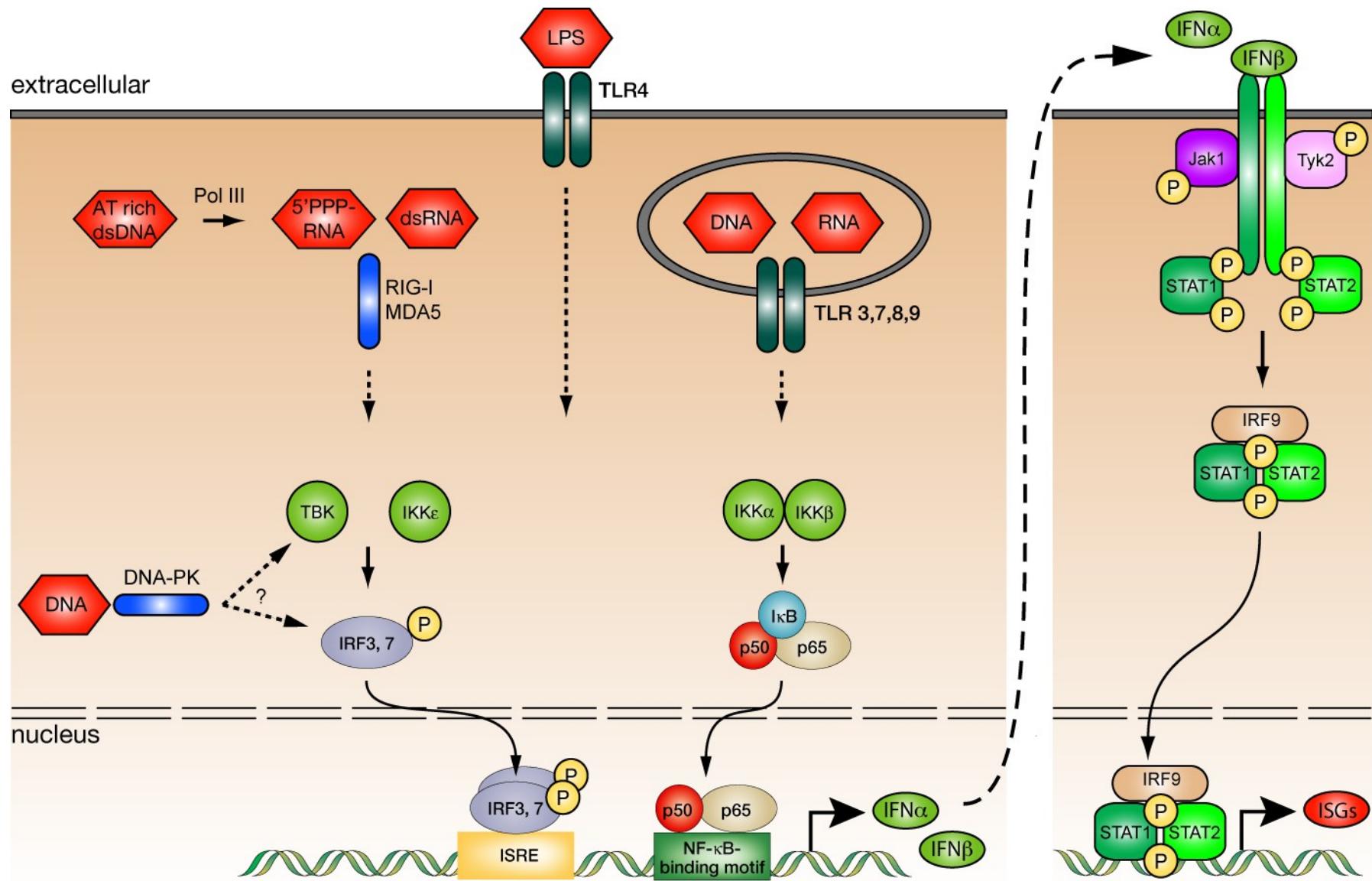
# How cells sense DNA in their cytosol



# Control of NF-κB activity



## How cells produce IFN $\alpha$ / $\beta$ and how IFN signals



# Cell-autonomous anti-viral effectors mediate the anti-viral state

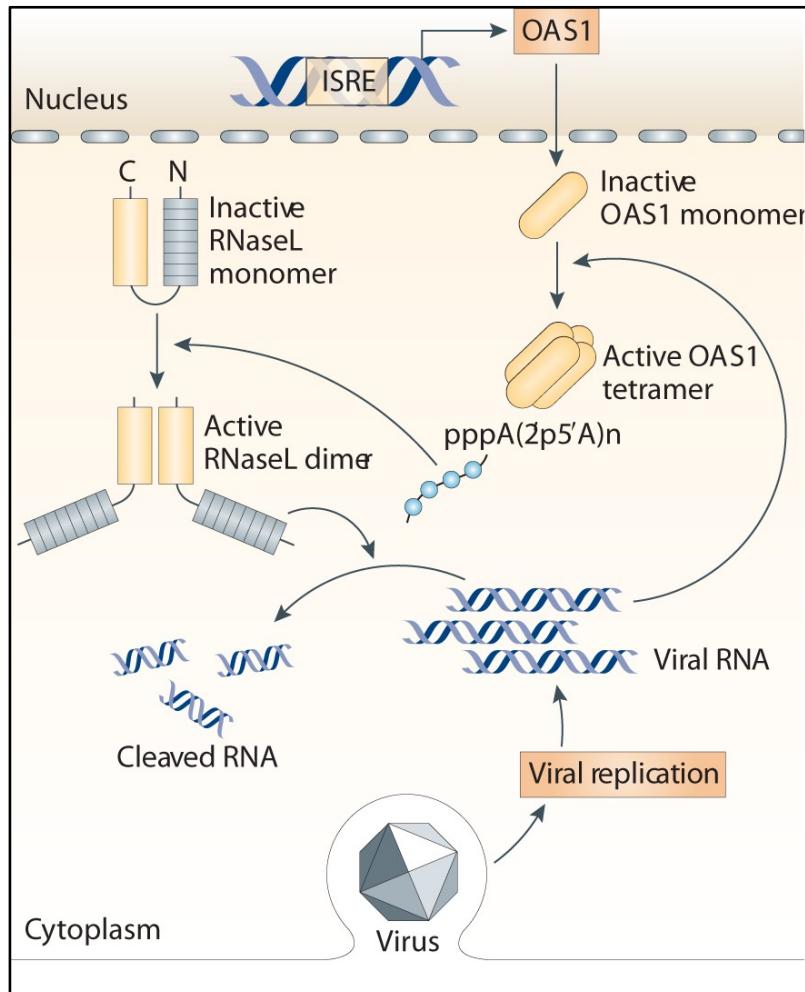
many mechanisms induced / enhanced by IFN => 'the antiviral state' of a cell, mediated by ISGs  
(interferon-stimulated genes)

=> many viral processes affected

- 
- degradation of viral genomic RNA RNaseL and OAS
  - hypermutating the viral genome APOBEC3G
  - inhibition of translation PKR
  - interference with viral protein function ISGylation
  - trapping of viral capsids MxA
  - inhibition of budding tetherins

# Cell-autonomous anti-viral effector mechanisms

OAS = 2' 5' oligoadenylate synthetase and RNaseL



=> degradation of viral RNA

OAS constitutively expressed (but IFN-upregulated)  
-> PRR function

RNaseL constitutively expressed

cleaved RNA feeds forward into RIG1 / MDA5 pathway

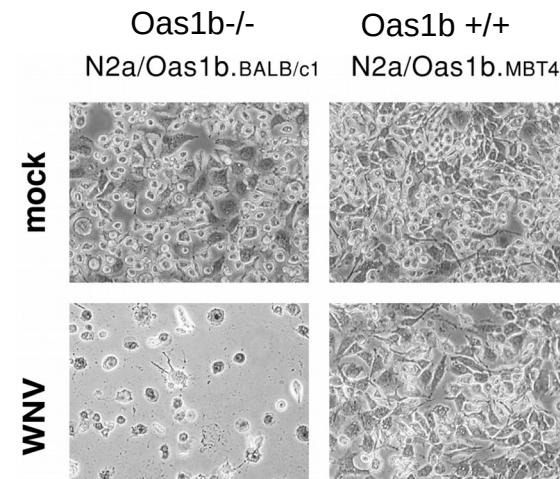
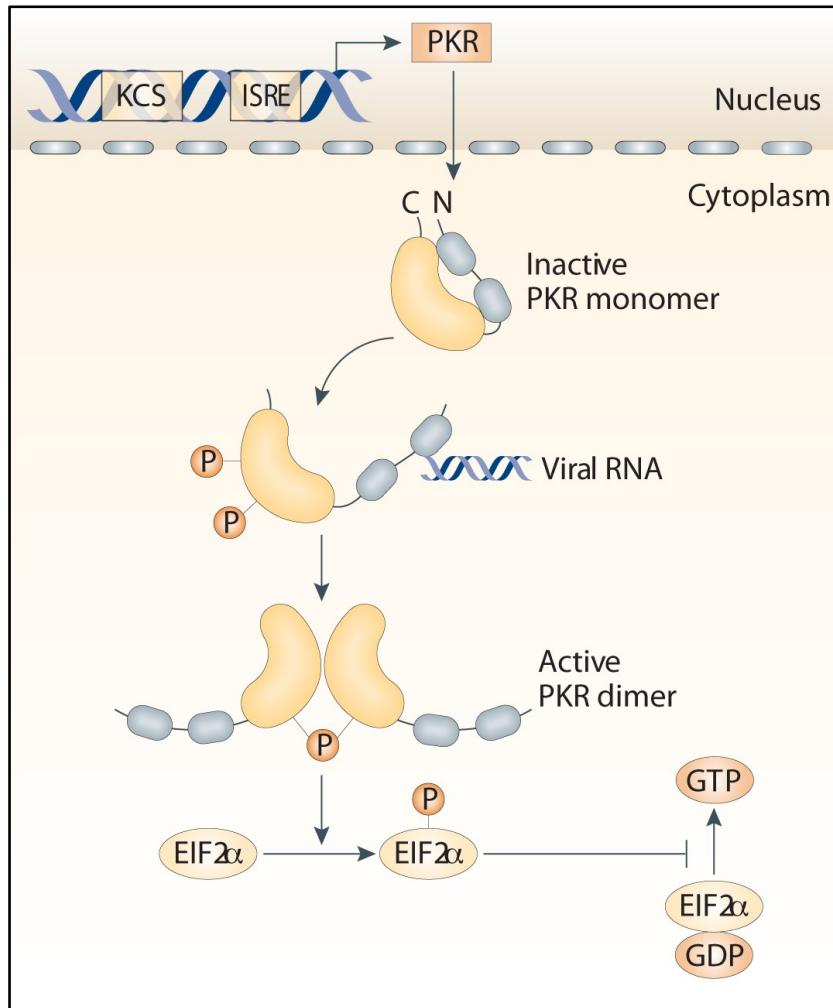


Figure 6 Enforced expression of Oas1b provides protection against West Nile virus (WNV) infection. Stable cell lines N2a/Oas1b.BALB/c 1 and N2a/Oas1b.MBT 4 were infected with multiplicity of infection (MOI) of 0.01 of IS-98-ST1 (WNV) or mock-infected (mock). Cytopathic effects (CPE) were observed at 72 h postinfection. Magnification $\times$  400.

# Cell-autonomous anti-viral effector mechanisms

PKR = Protein Kinase R (R for RNA)



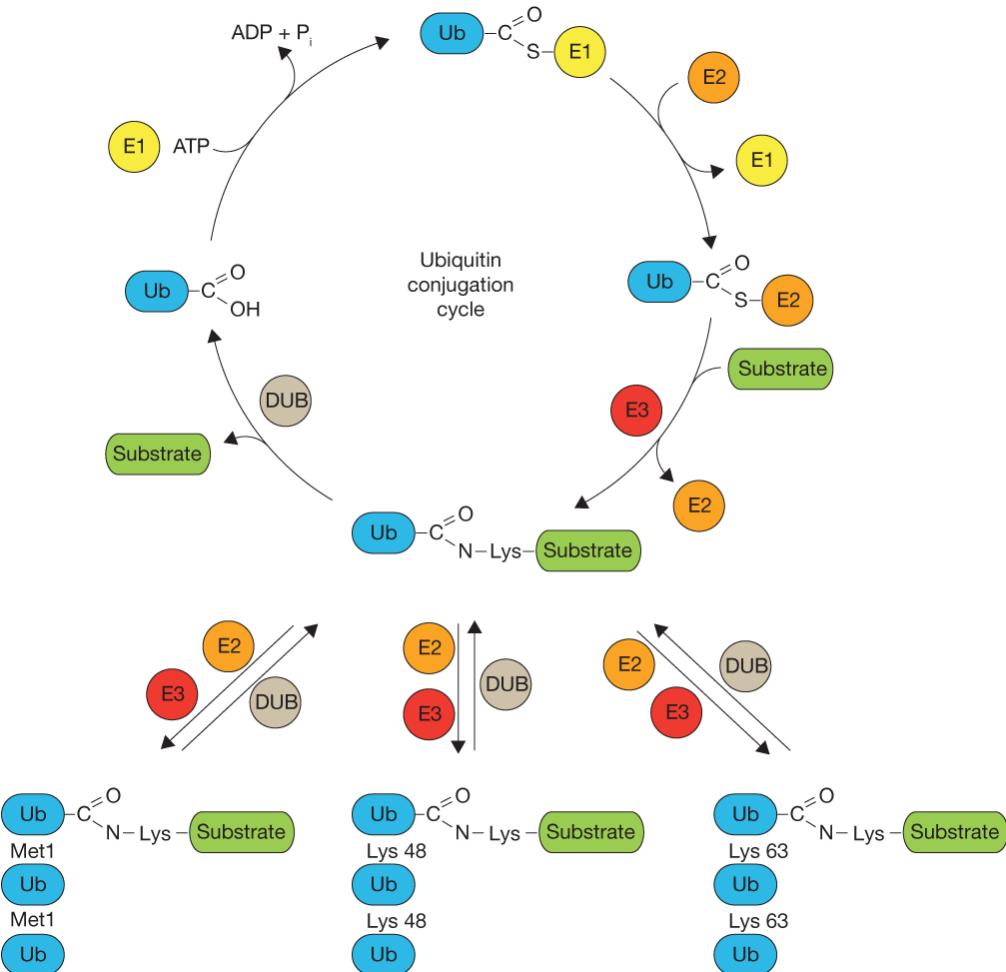
=> inhibits cap-dependent translation

constitutively expressed (but IFN-induced)  
-> PRR function

dimeric PKR activated by dsRNA above critical length  
->ruler function, similar to TLR3

# Cell-autonomous anti-viral effector mechanisms

Intermezzo: Conjugation / de-conjugation of ubiquitin and ubiquitin-like proteins (UBLs)



superfamily of small, distantly related proteins  
ubiquitin, ISG15, ATG8/LC3 ...

posttranslationally conjugated to  
primary aminofunctions of proteins, i.e. Lys or N-terminus  
phosphatidylethanolamine (ATG8/LC3)

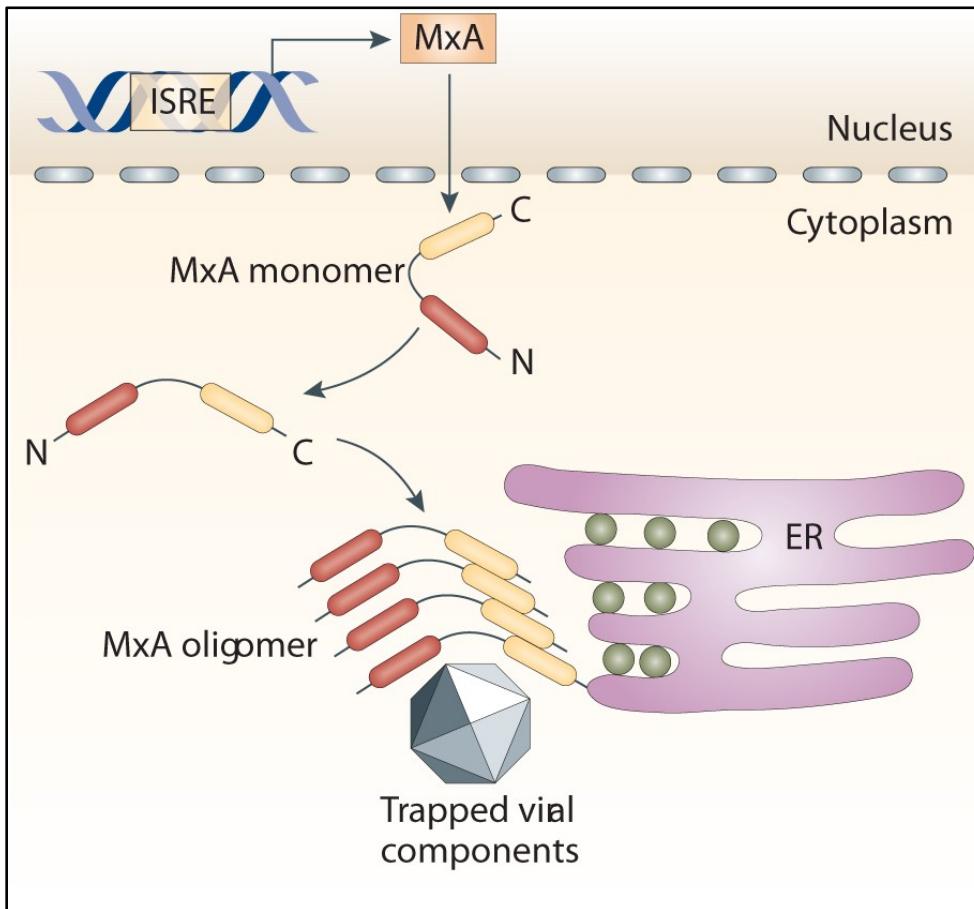
conjugation requires  
E1 – ub activating enzyme  
E2 – ub conjugating enzyme  
E3 – ub ligase

ubiquitin and Sumo are conjugated into chains:  
one chain type for Sumo  
eight for ubiquitin

reversible modification, i.e. deconjugation by isopeptidases

# Cell-autonomous anti-viral effector mechanisms

MxA = myxovirus resistance locus

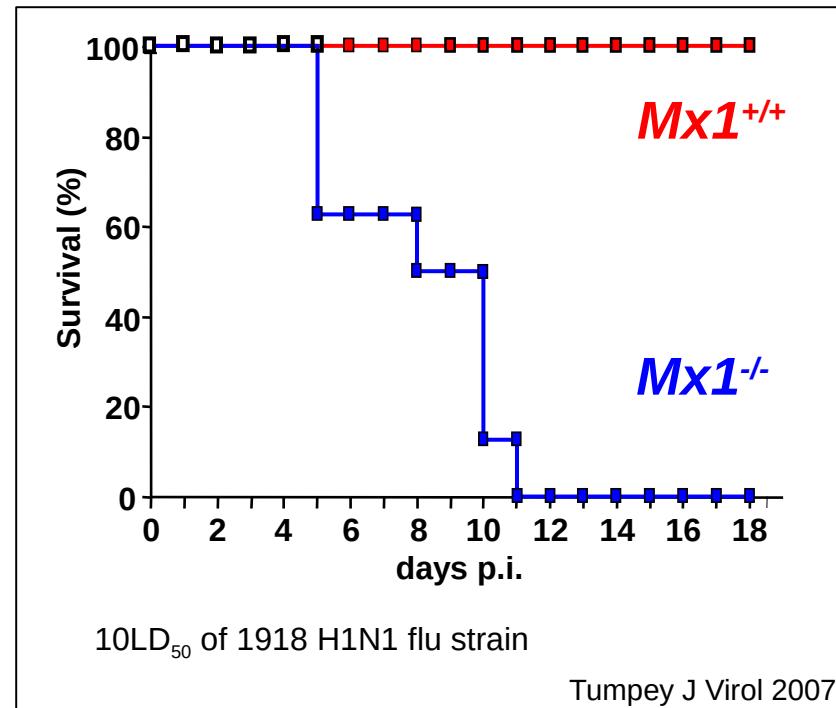


=> mutation in Mx1 causes susceptibility of most mouse strains to influenza

GTPase

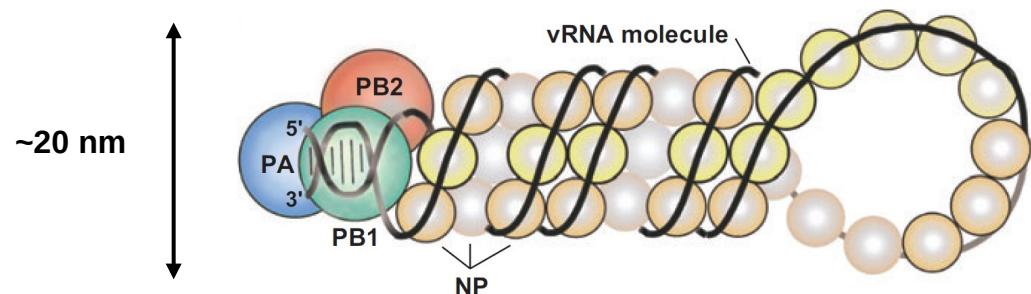
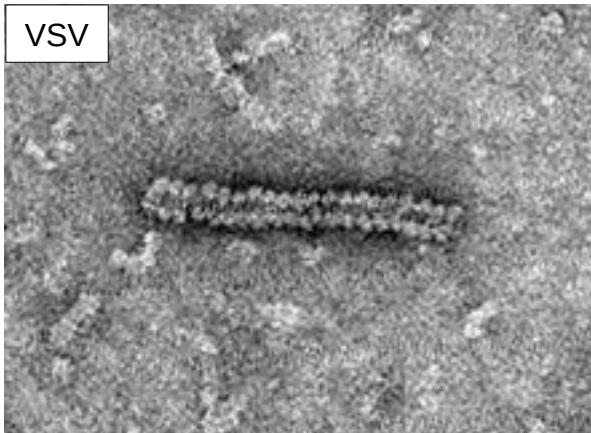
assembles into oligomers,  
detects viral assemblies (capsids?), and  
interferes with viral replication / exit

receptor and effector mechanism largely unclear



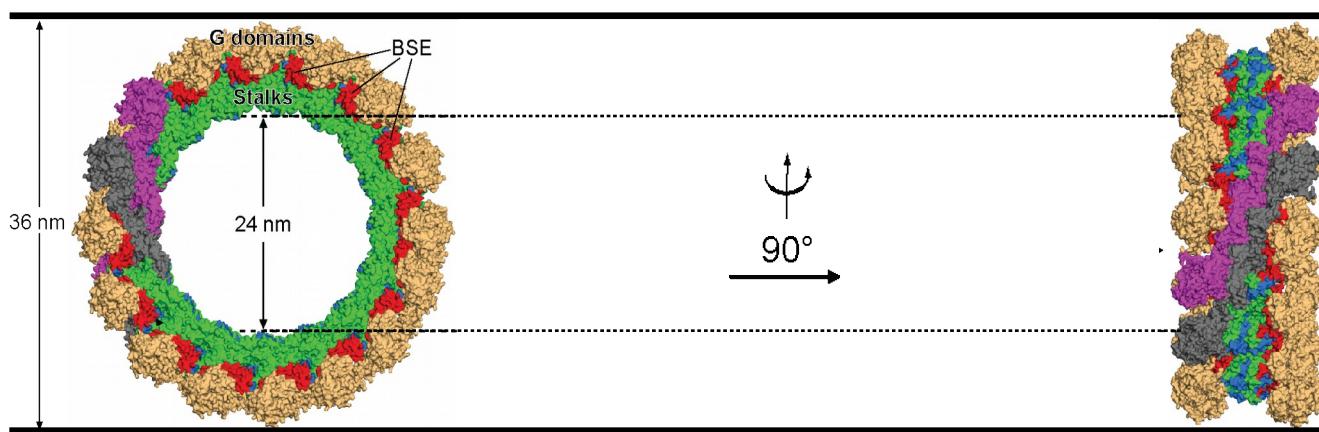
# Cell-autonomous anti-viral effector mechanisms

MxA = myxovirus resistance locus



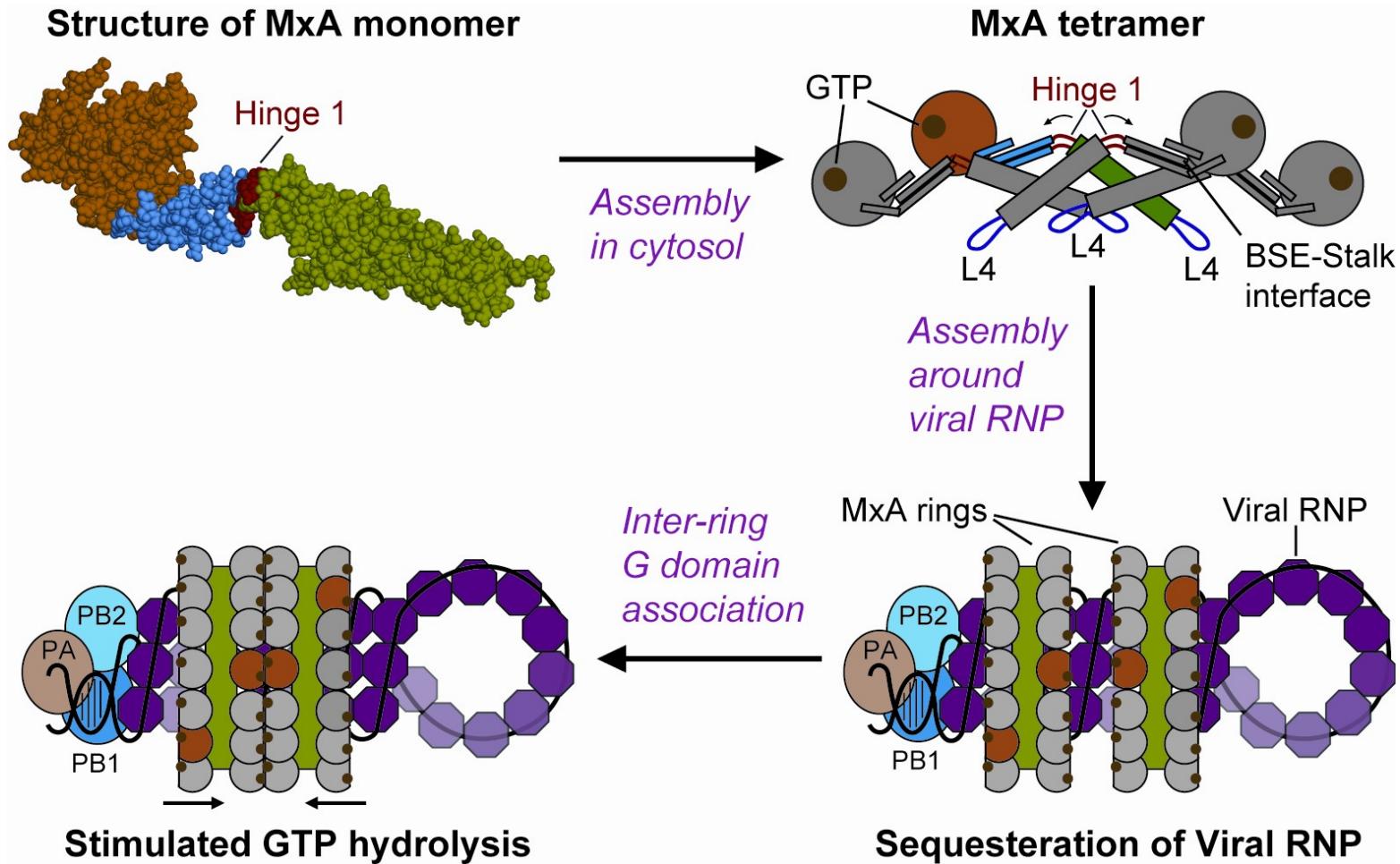
Naffakh, N. et al. Annu. Rev. Microbiol. 2008. 62:403–424

## vRNPs (nucleocapsids)



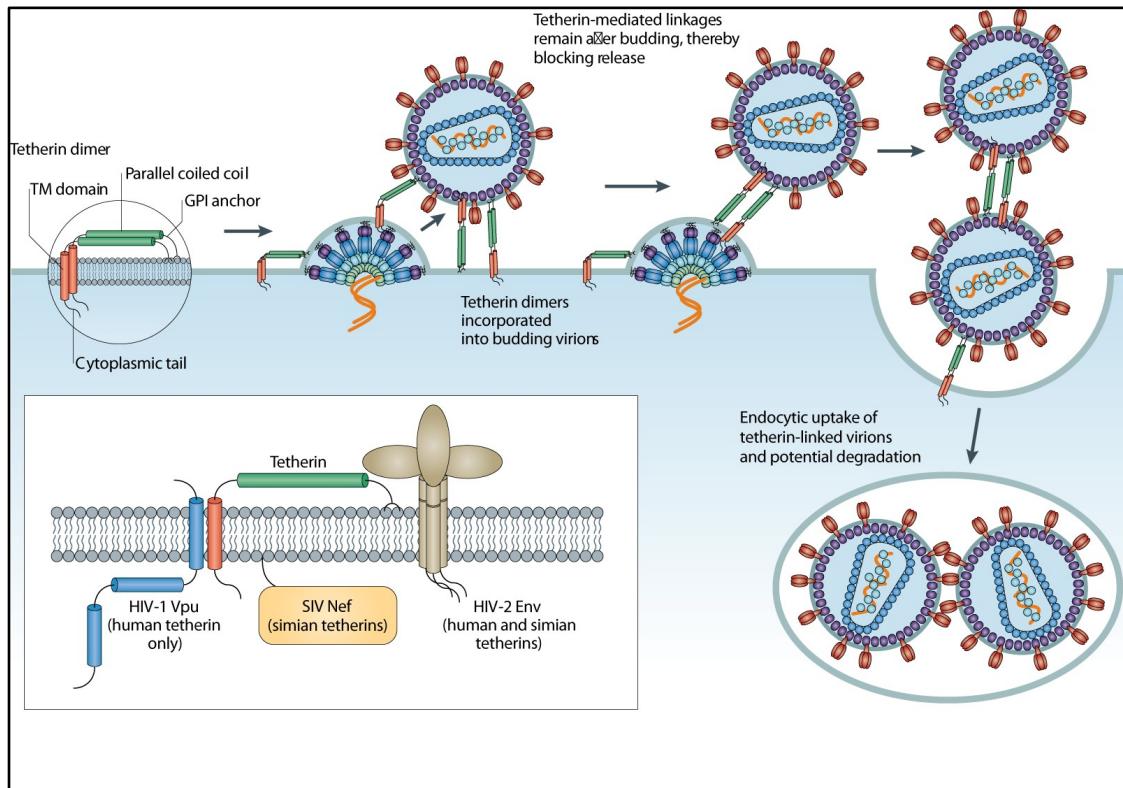
# Cell-autonomous anti-viral effector mechanisms

MxA = myxovirus resistance locus



# Cell-autonomous anti-viral effector mechanisms

## Tetherin



- => antagonizes virion release
- broad activity against enveloped viruses
- immutable target requires active viral antagonism
- SIV: Nef  
antagonism depends on G/DDIWK motif in tetherin  
motif lacking in human tetherin
- HIV1: VPU  
intracellular retention of tetherin  
recruitment of SCF $\beta$ TRCP -> ub-dependent degradation
- HIV2: Env

If you were a pathogen, where would you live?

extracellular

or

intracellular

vesicles

or

cytosol

immunity



nutrients

## If you were a pathogen, where would you live?

extracellular

or

intracellular

vesicles

or

cytosol

immunity

antibodies  
complement  
phagocytes

nutrients

variable



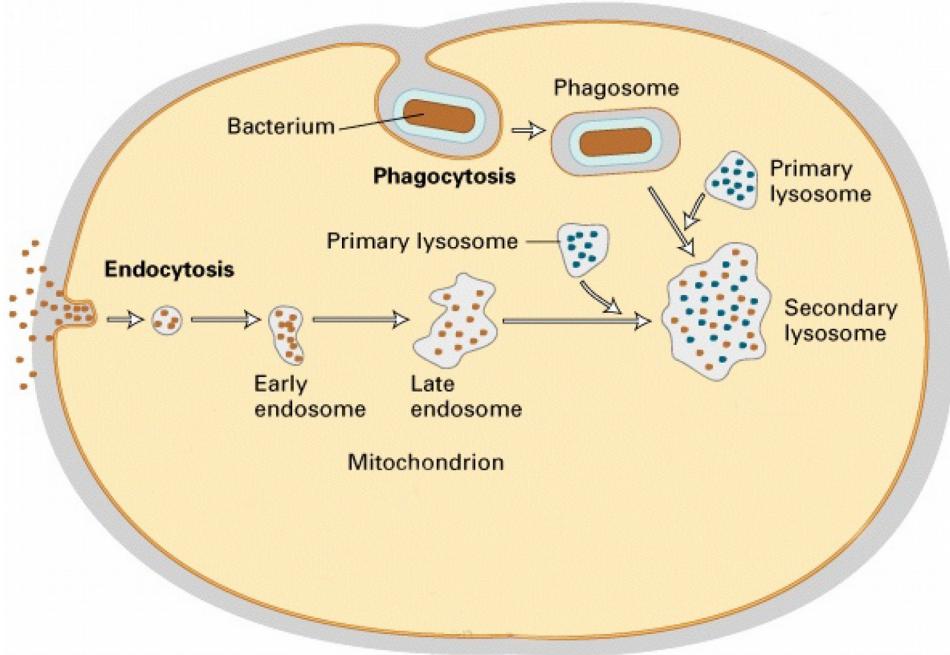
## If you were a pathogen, where would you live?

	extracellular	or	intracellular		
			vesicles	or	cytosol
immunity	antibodies complement phagocytes		low pH hydrolytic enzymes radicals		autophagy
nutrients	variable		low		high

Brucella  
Chlamydia  
Legionella  
Leishmania  
Mycobacteria  
Plasmodium  
Salmonella  
Toxoplasma  
Wolbachia  
etc

Shigella  
Listeria  
Rickettsia  
*Trypanosoma cruzii*

## Lysosomes kill phagocytosed bacteria and keep the vesicular compartment sterile



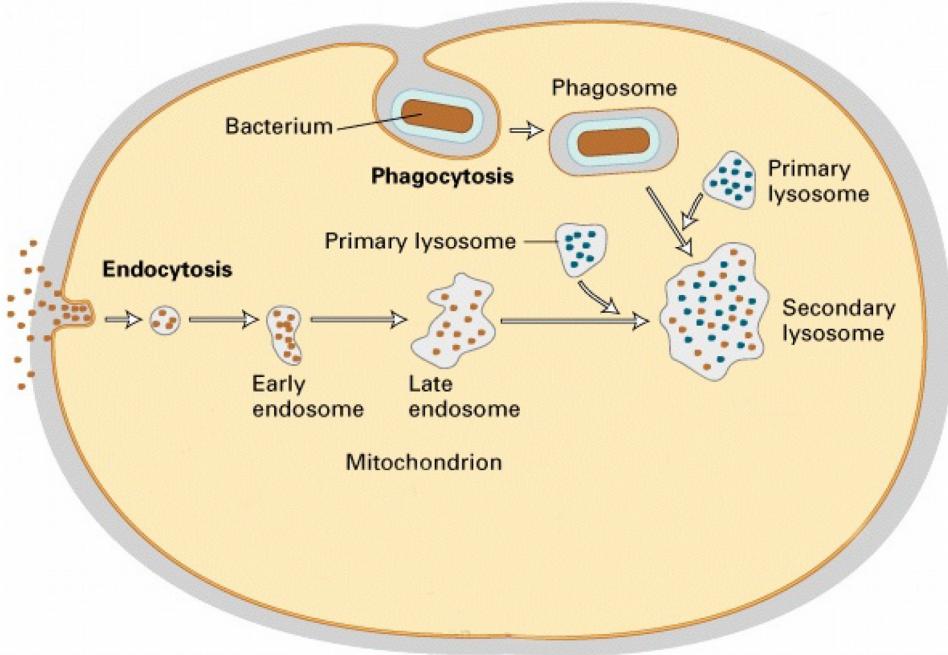
neutrophil + E. coli

neutrophil + Streptococcus pyogenes

### Lysosomal effector mechanisms

- low pH
- hydrolytic enzymes
  - glycosidases (lysozyme)
  - proteases
  - lipases
- nutrient limitations (lactoferrin, NRAMP1)
- anti-bacterial peptides (defensins)
- radicals (NO, ROS)

## Lysosomes kill phagocytosed bacteria and keep the vesicular compartment sterile



neutrophil + S. aureus

neutrophil + Streptococcus pyogenes

### Lysosomal effector mechanisms

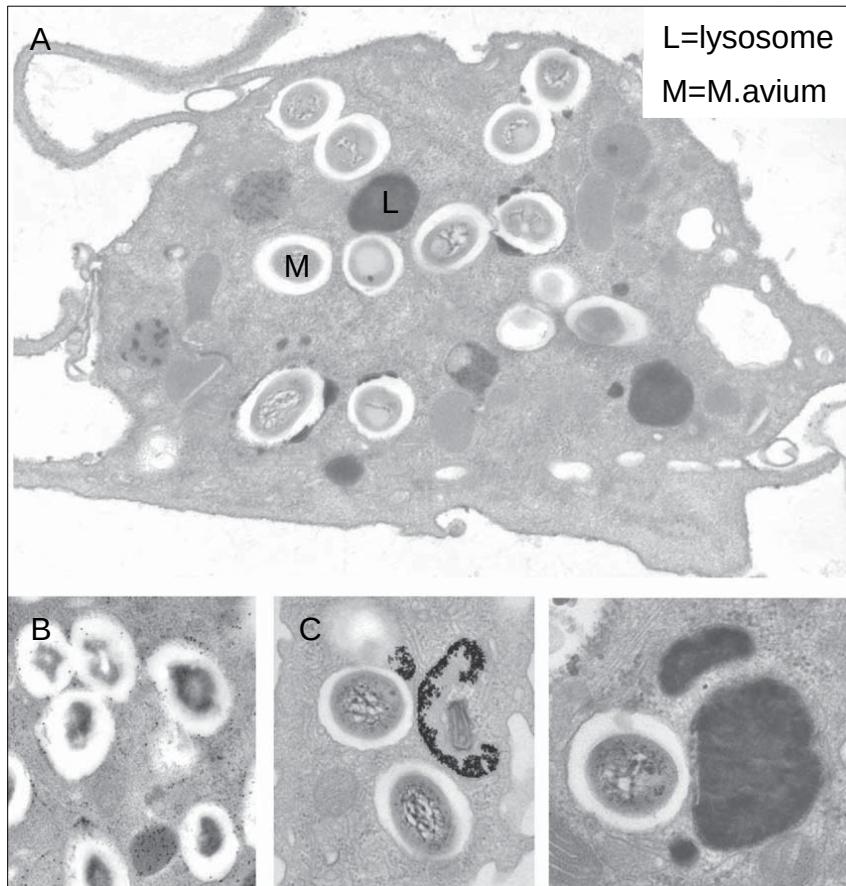
- low pH
- hydrolytic enzymes
  - glycosidases (lysozyme)
  - proteases
  - lipases
- nutrient limitations (lactoferrin, NRAMP1)
- anti-bacterial peptides (defensins)
- radicals (NO, ROS)

# Professional vesicle-dwelling pathogens avoid delivery to the lysosome

## Mycobacterium tuberculosis

1/3 of world population infected,  
1 new infection / second,  
2 Mio deaths / annum

mouse macrophage + Mycobacterium avium



- A) cells stained on day 14 p.i. with DAMP, a weak base, that accumulates in acidic compartments
- B) lysosomes stained for acid phosphatase on day 7 p.i.
- C) Cells incubated with BSA-gold, which first labels endosomes. Later, during a chase period, it accumulates in lysosomes.

=> note that vesicles containing bacteria are marker -ve

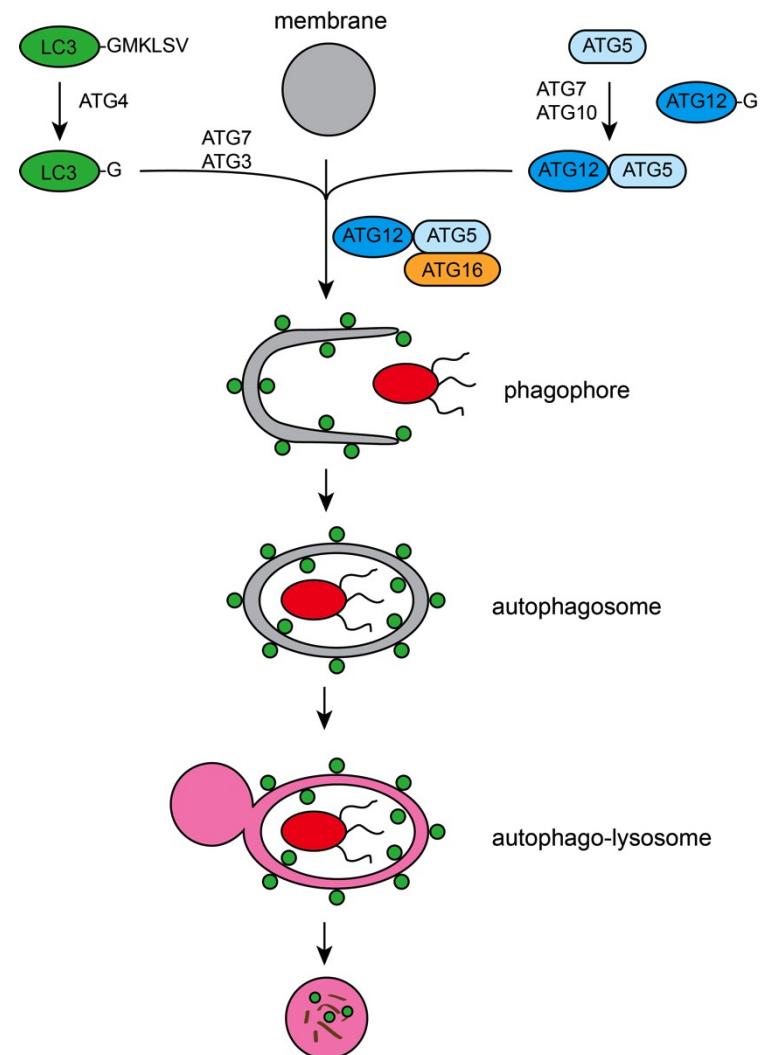
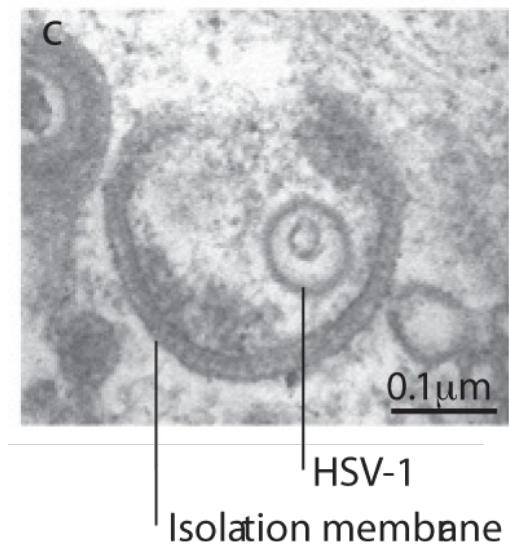
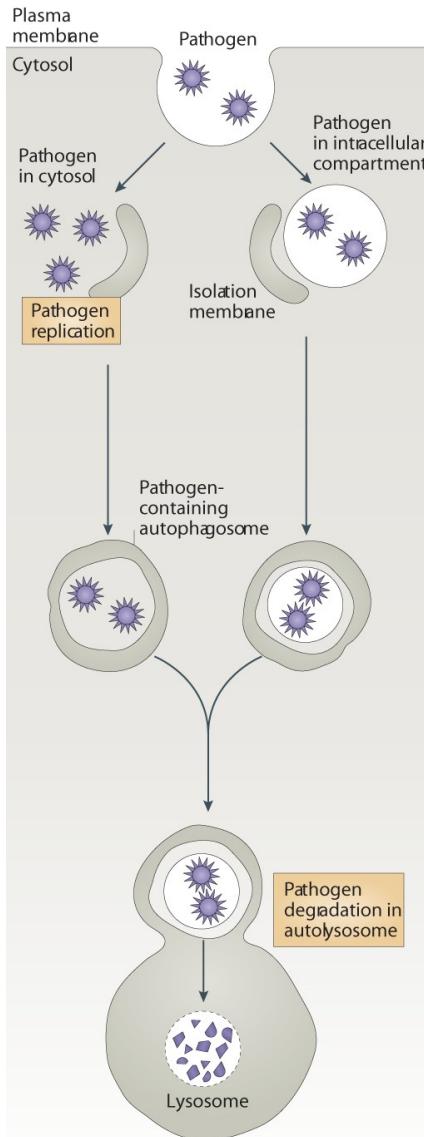
## If you were a pathogen, where would you live?

	extracellular	or	intracellular	
		vesicles	or	cytosol
immunity	antibodies complement phagocytes		low pH hydrolytic enzymes radicals	autophagy
nutrients	variable		low	high

Brucella  
Chlamydia  
Legionella  
Leishmania  
Mycobacterium (except marinum)  
Plasmodium  
Salmonella  
Toxoplasma  
Wolbachia  
etc

Shigella  
Listeria  
Rickettsia  
*Trypanosoma cruzii*

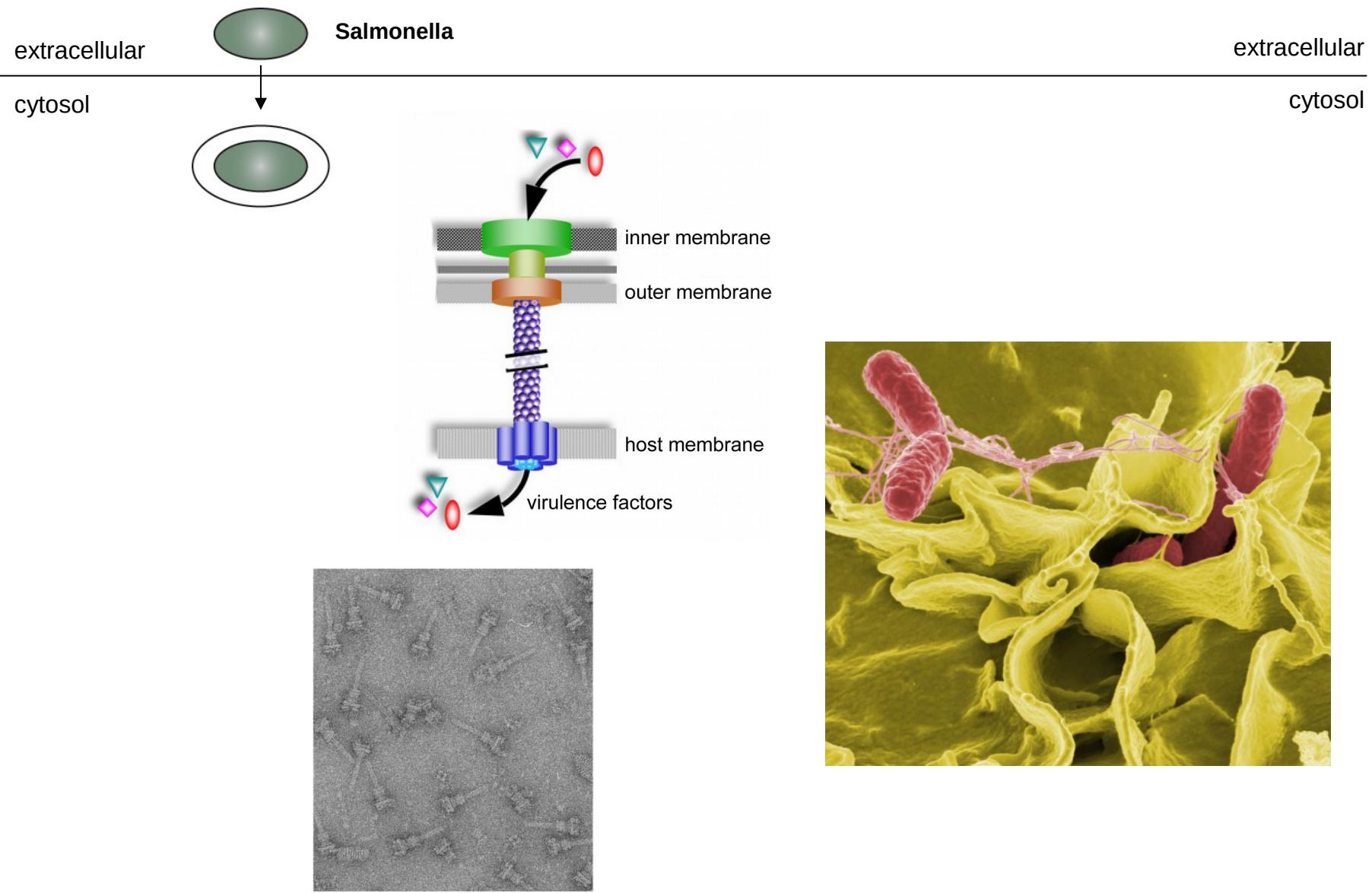
# Autophagy defends the cytosol against bacterial invasion



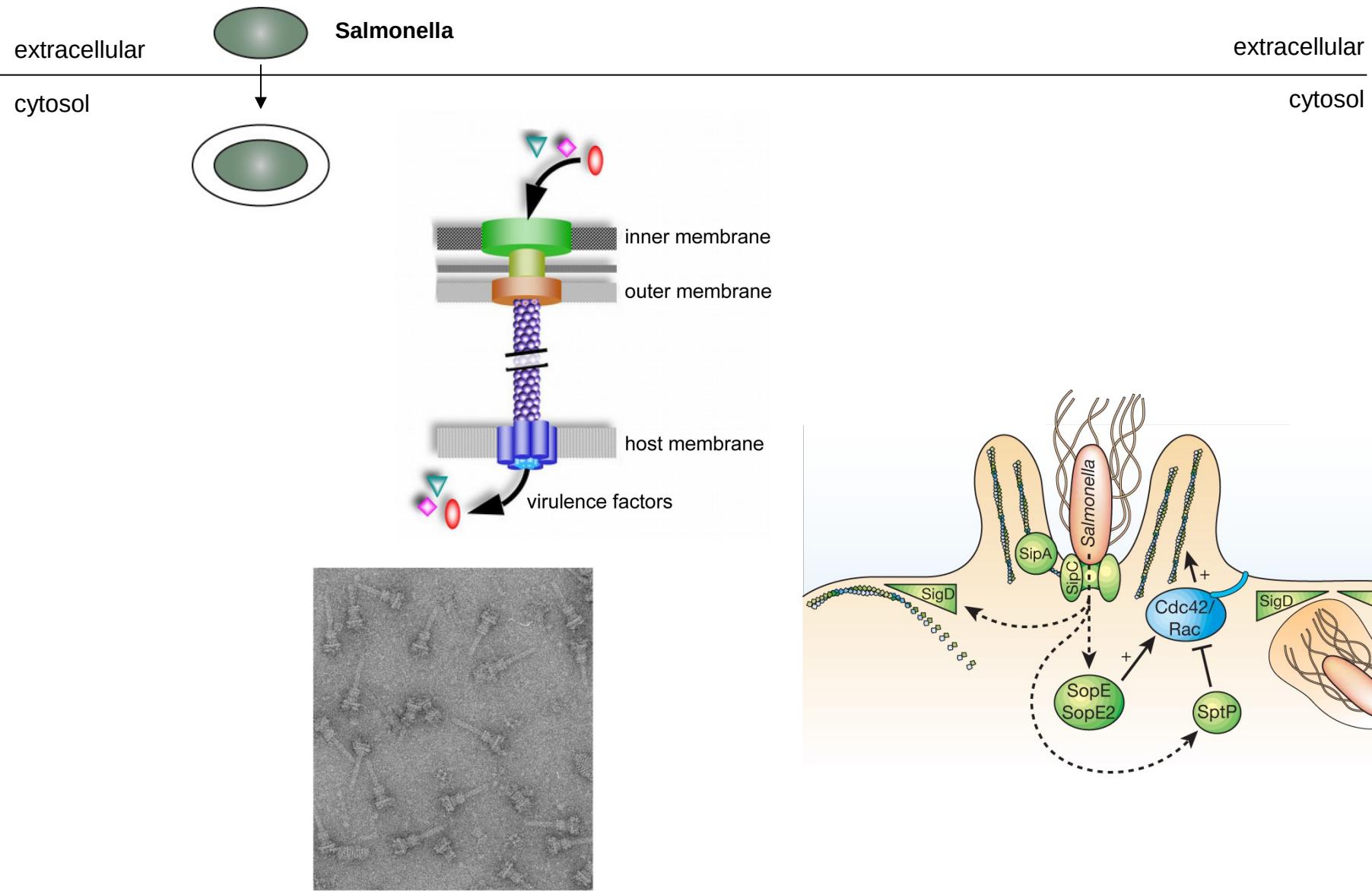
## Cytosolic immunity against invading bacteria



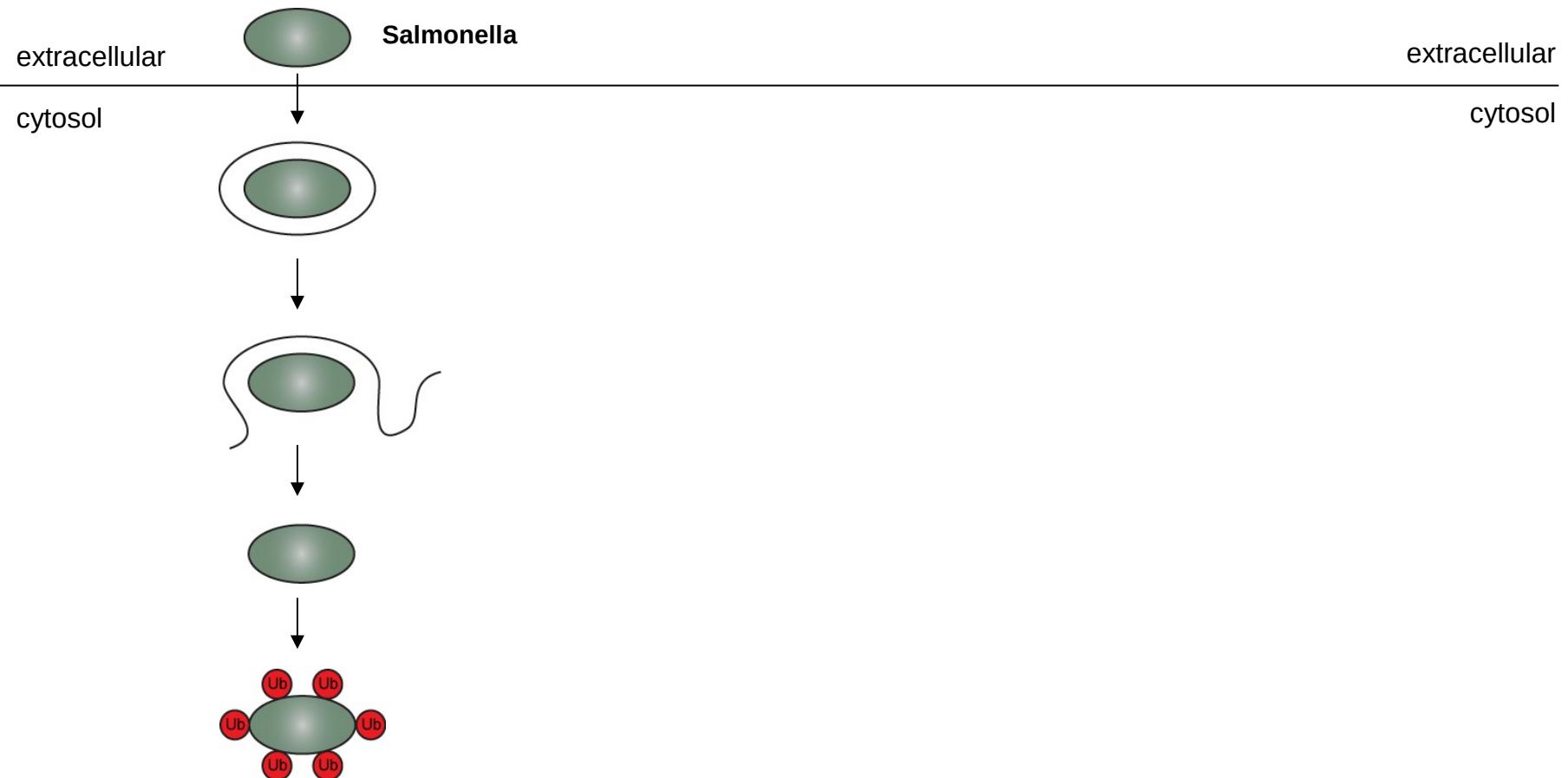
# Cytosolic immunity against invading bacteria



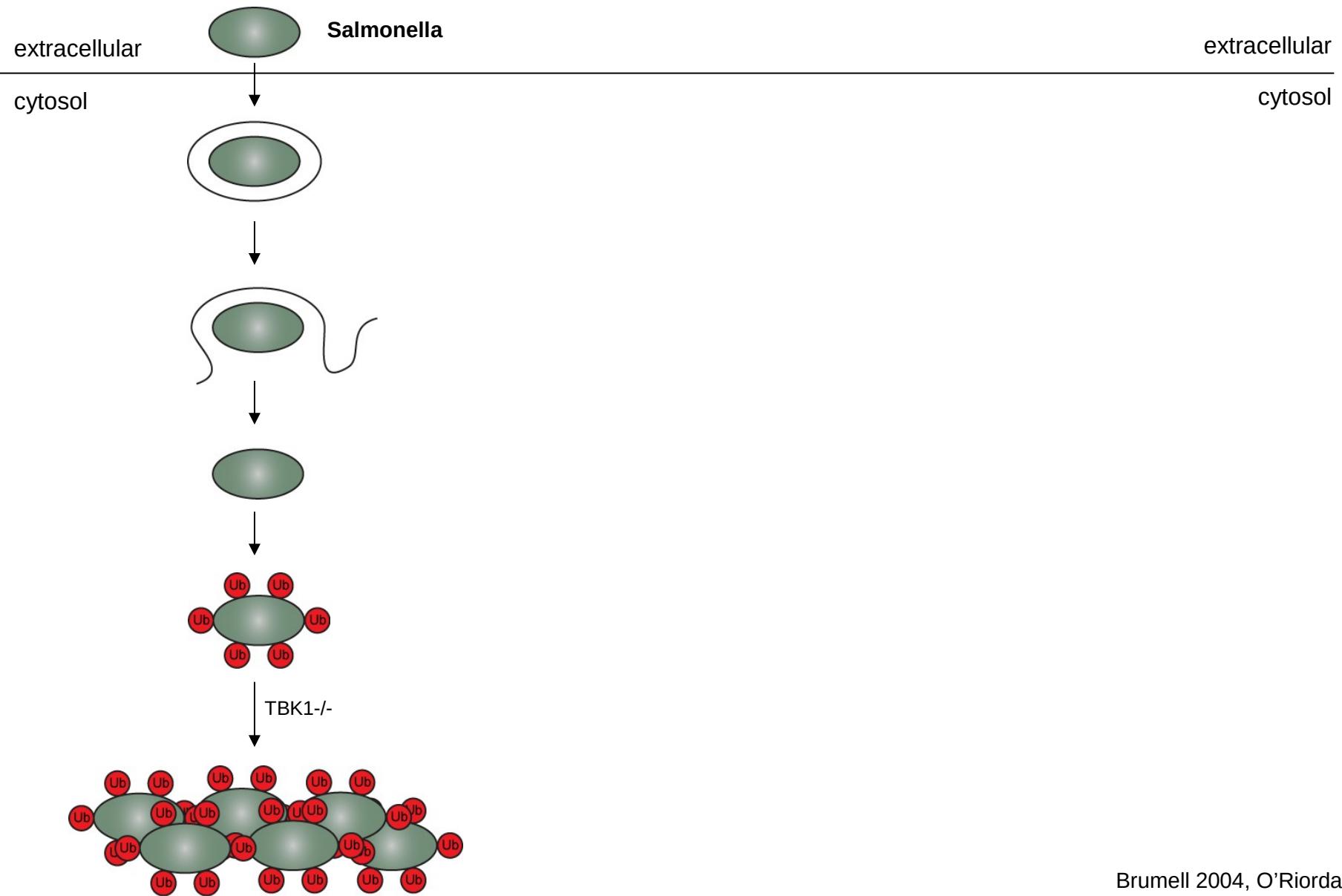
# Cytosolic immunity against invading bacteria



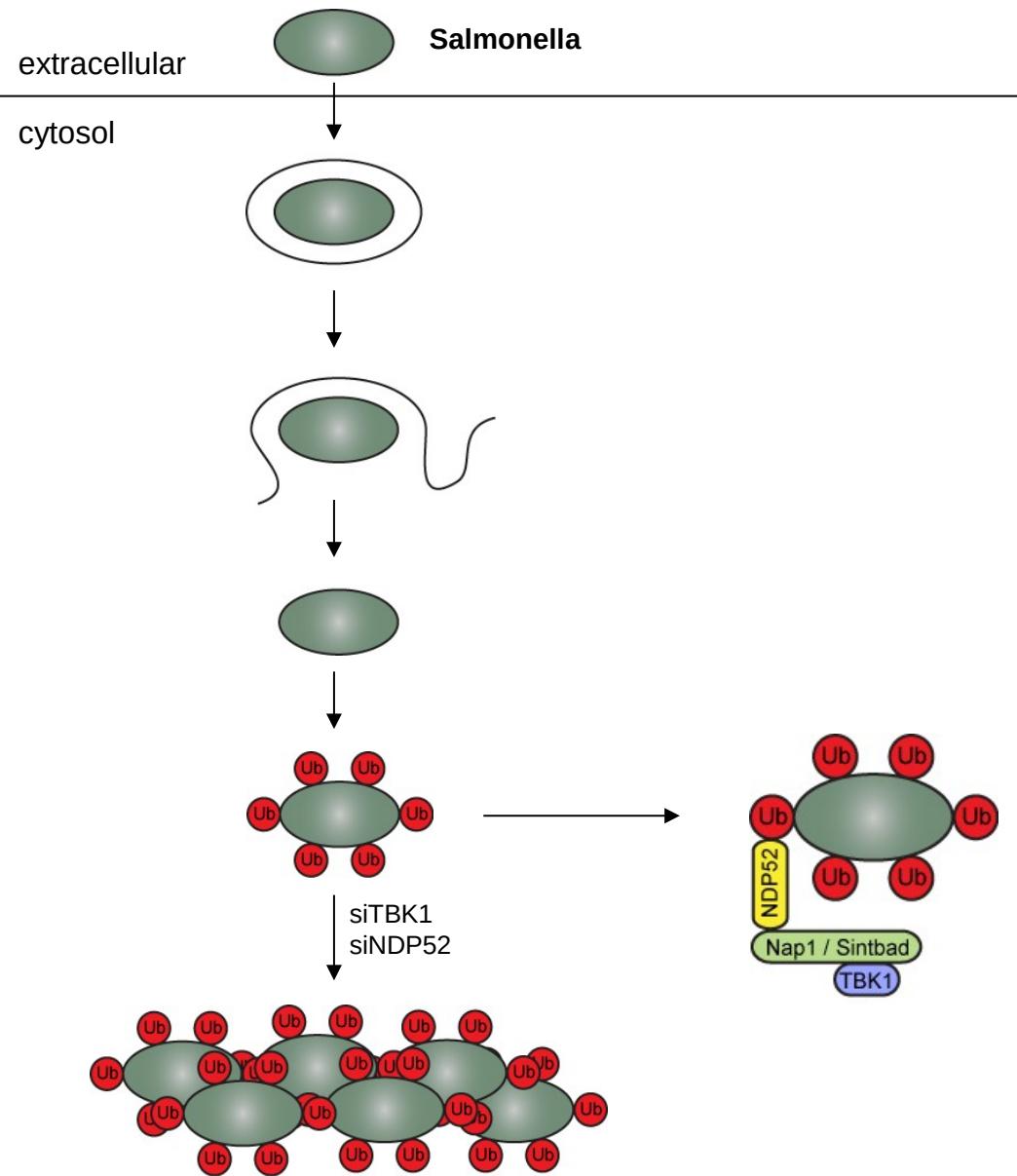
## Cytosolic immunity against invading bacteria



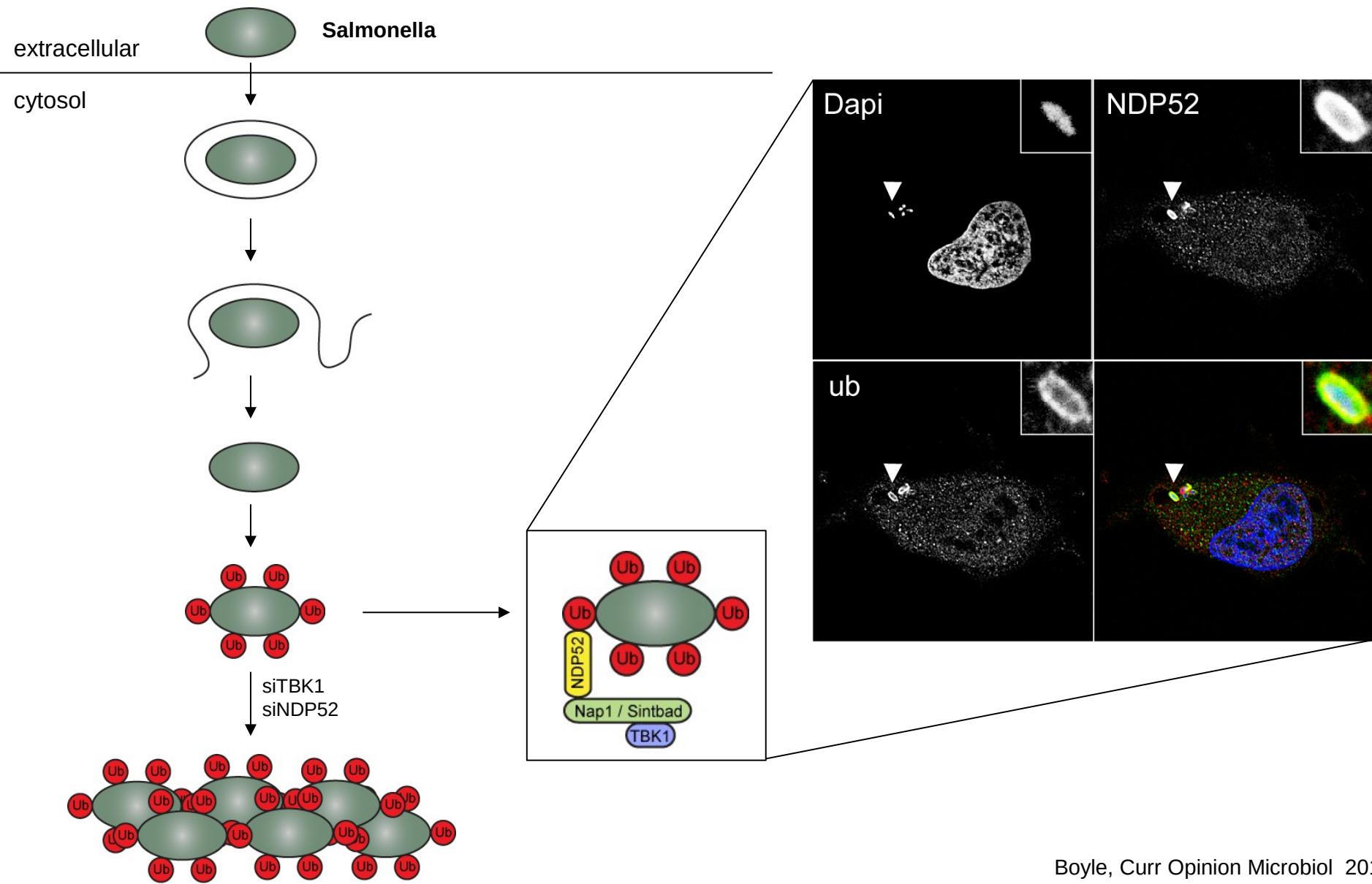
## Cytosolic immunity against invading bacteria



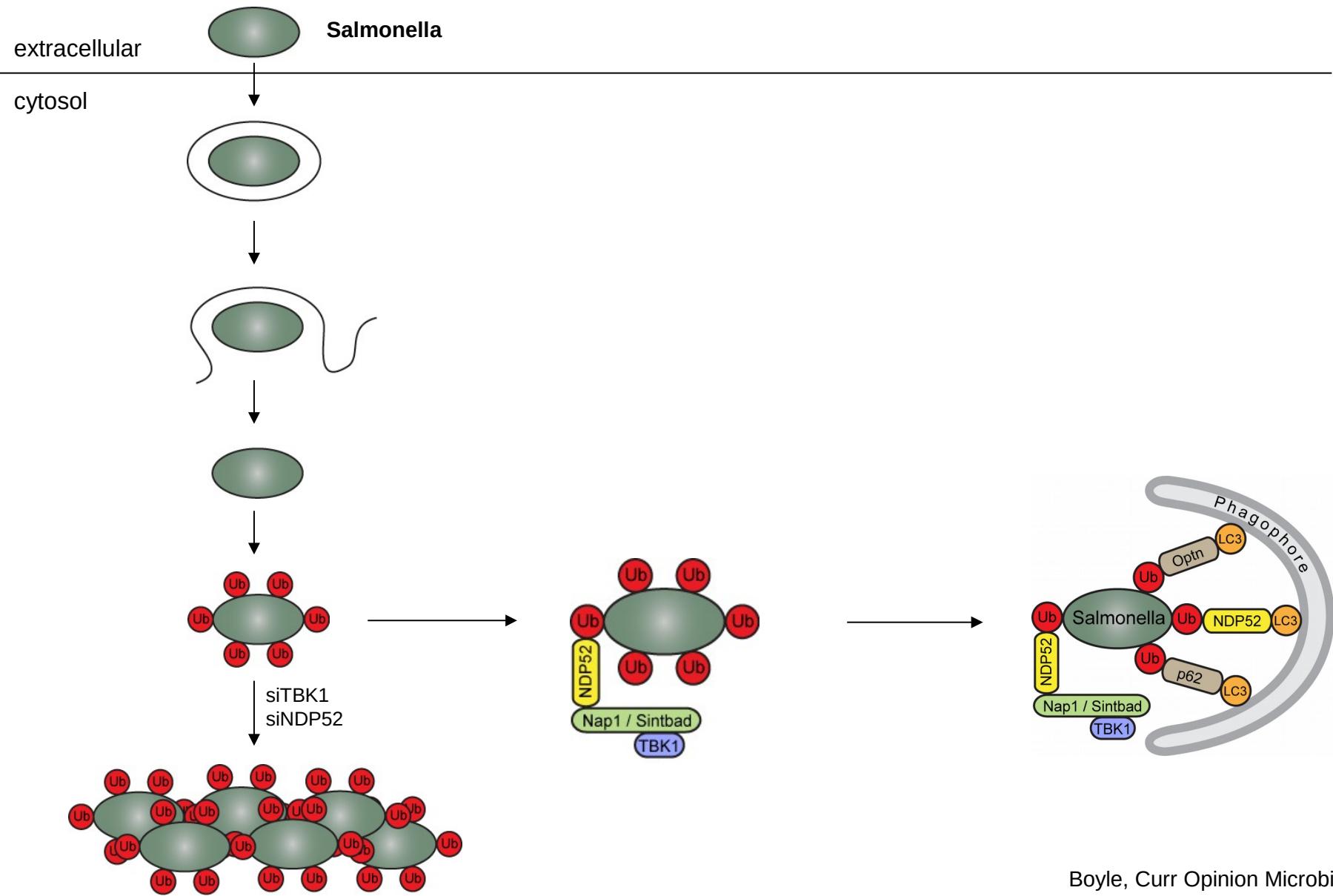
## The ubiquitin / autophagy model of cytosolic defence



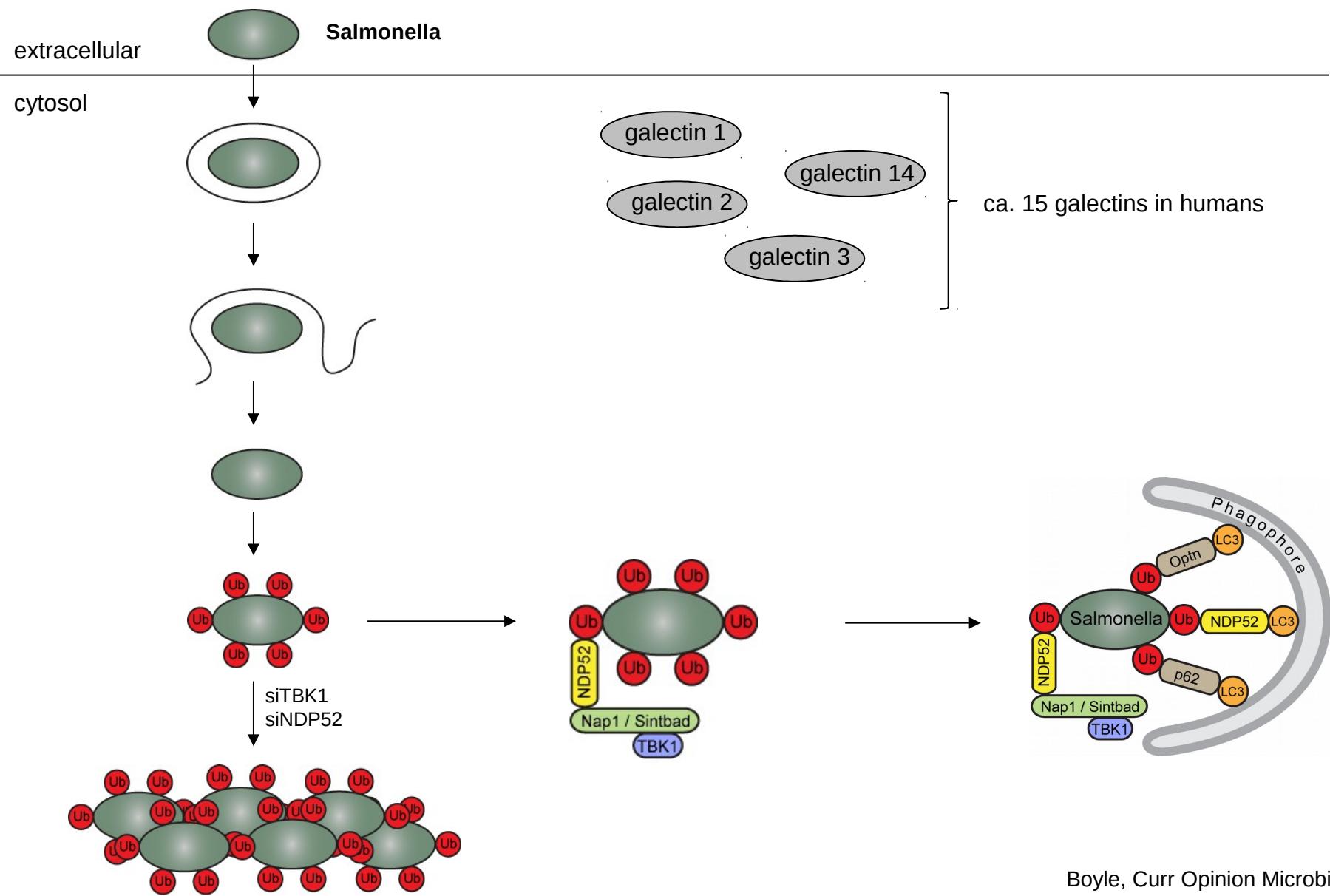
# The ubiquitin / autophagy model of cytosolic defence



# The ubiquitin / autophagy model of cytosolic defence



## A novel, danger-sensing pathway to catch bacteria



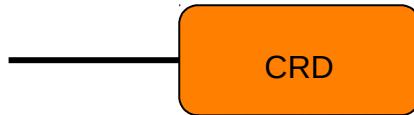
# Galectins - a family of cytosolic lectins in search of a function

**single CRD**



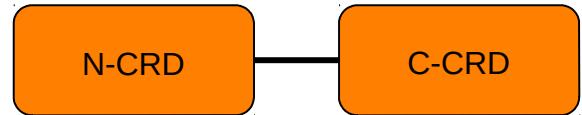
Galectins 1, 2, 7, 10, 13, 14  
HSPC159

**extended CRD**



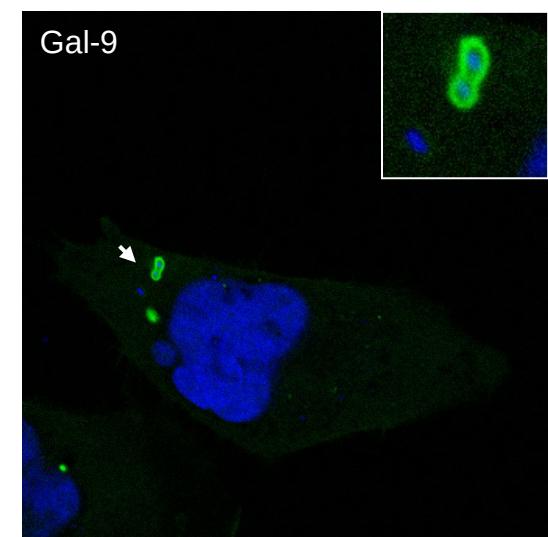
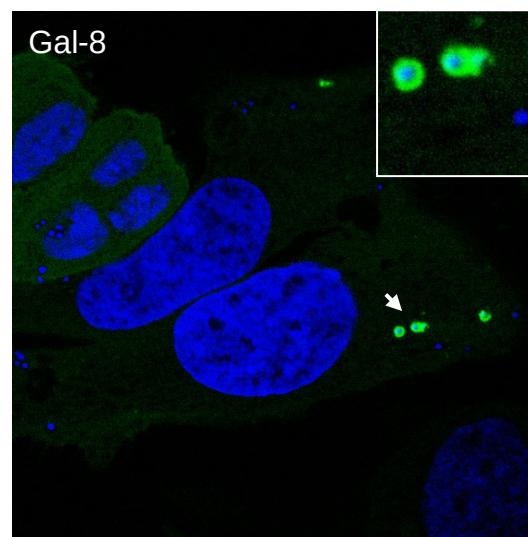
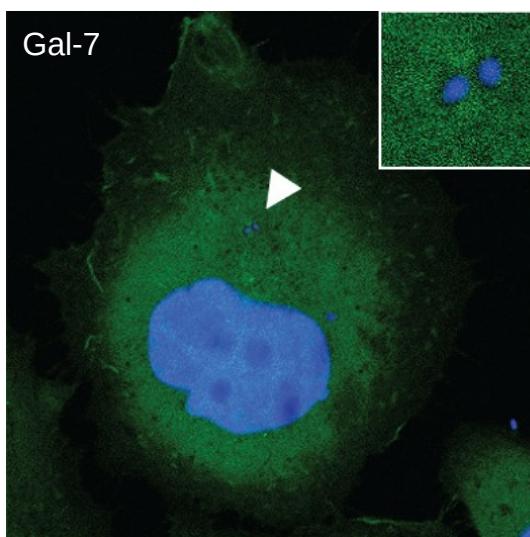
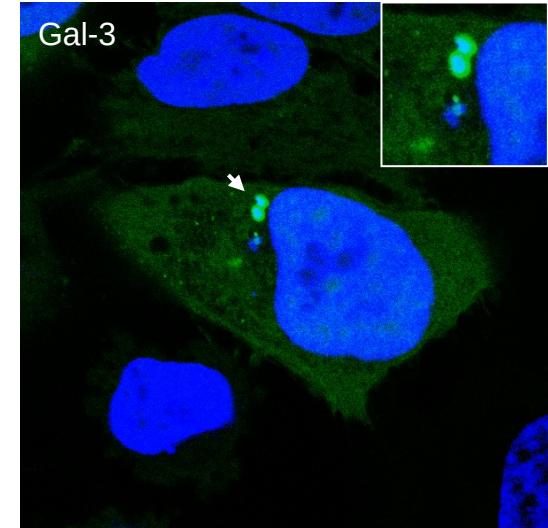
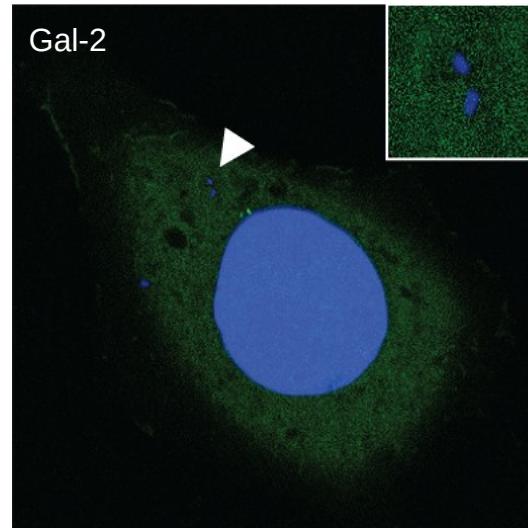
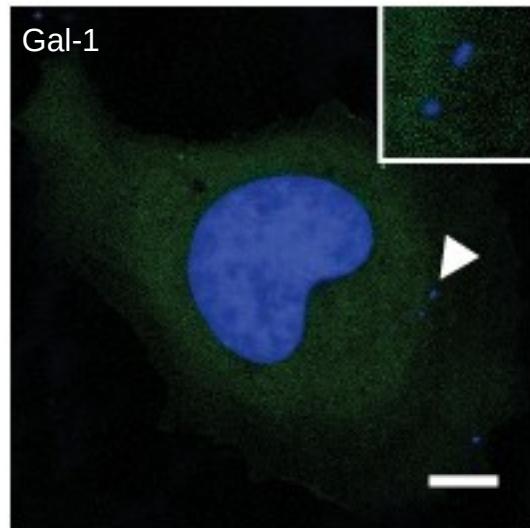
Galectin 3

**double CRD**

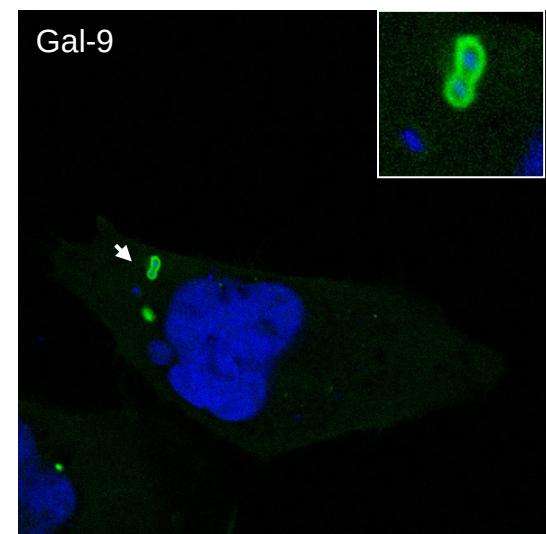
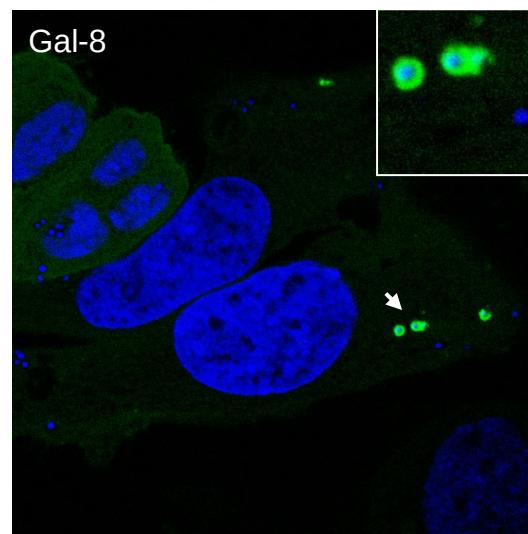
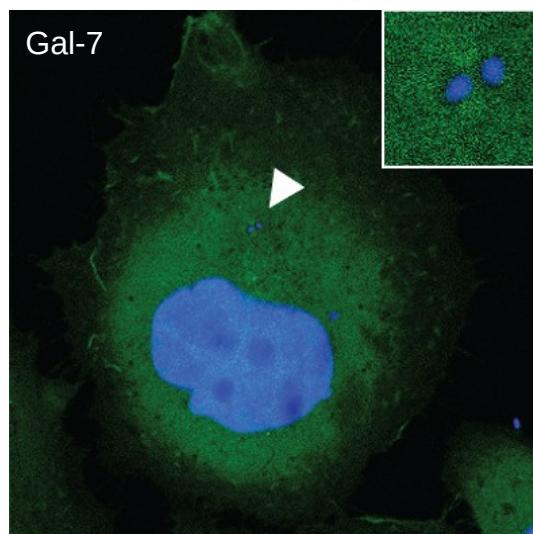
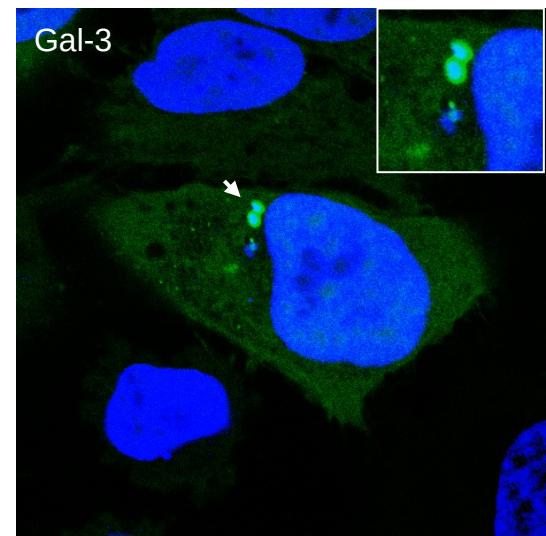
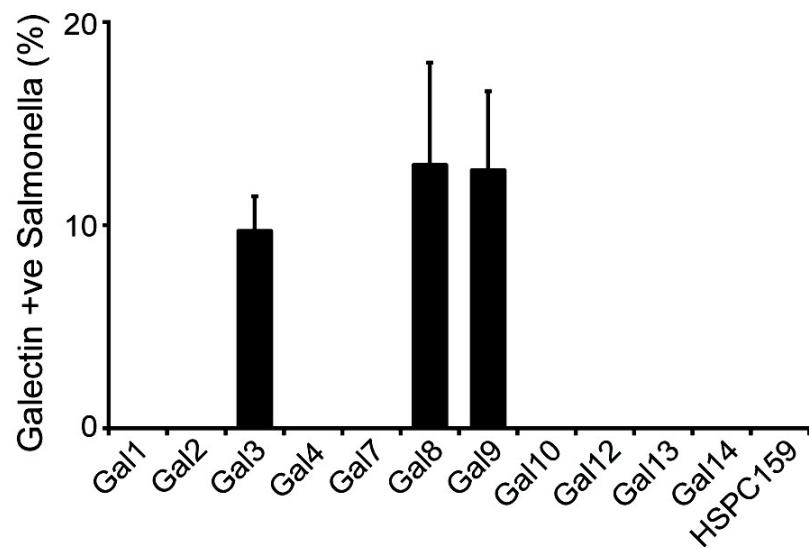


Galectins 4, 8, 9, 12

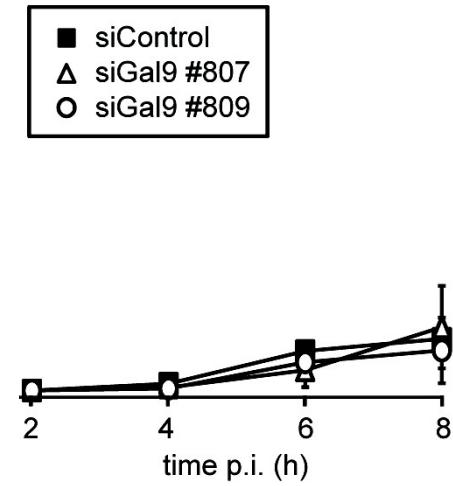
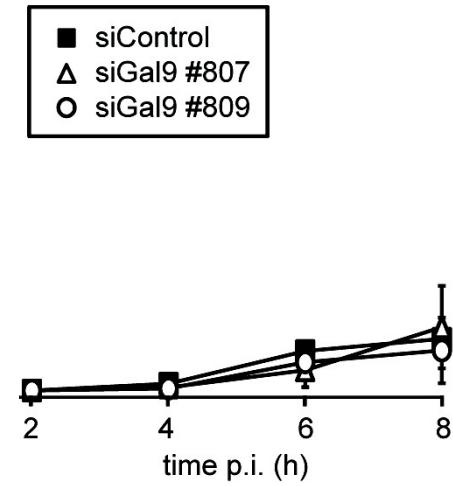
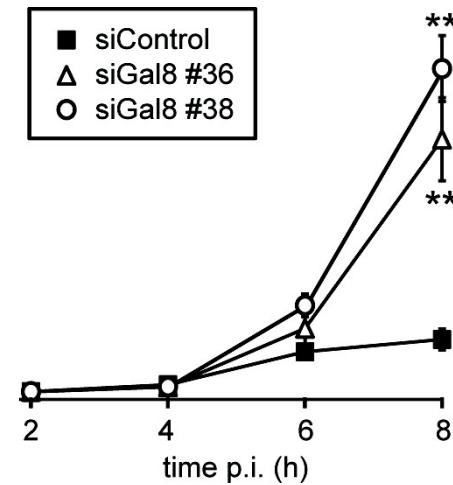
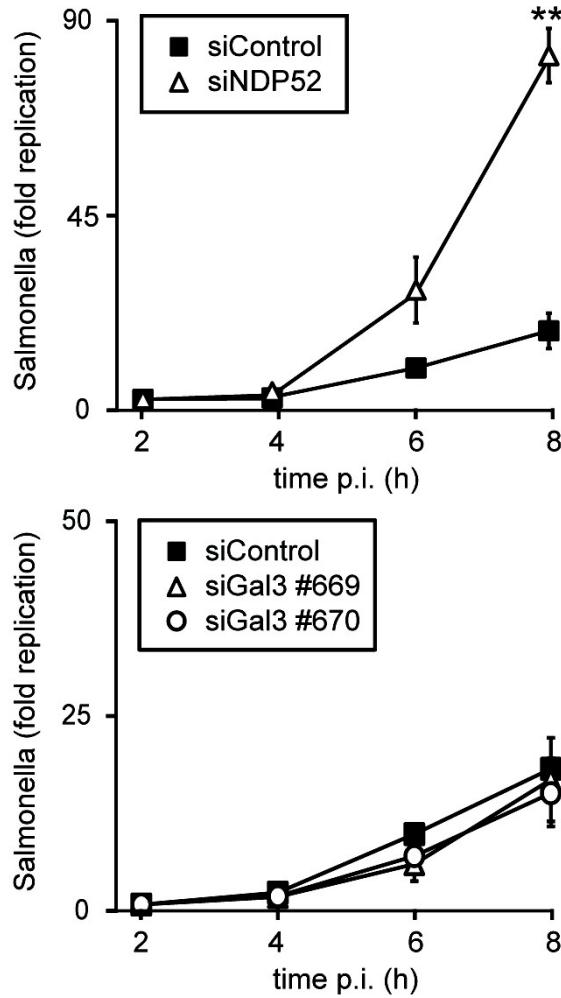
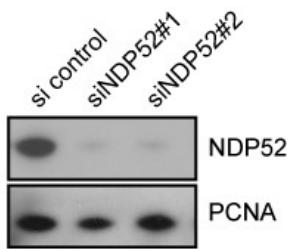
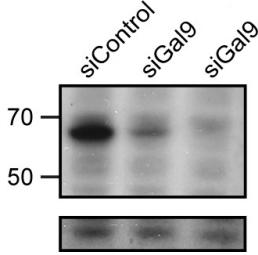
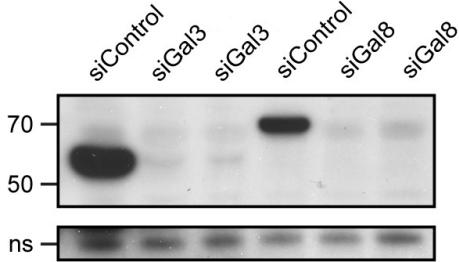
## Galectin-3, 8, and 9 detect invading Salmonella



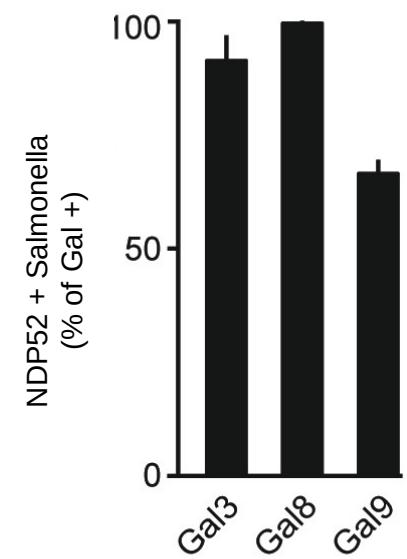
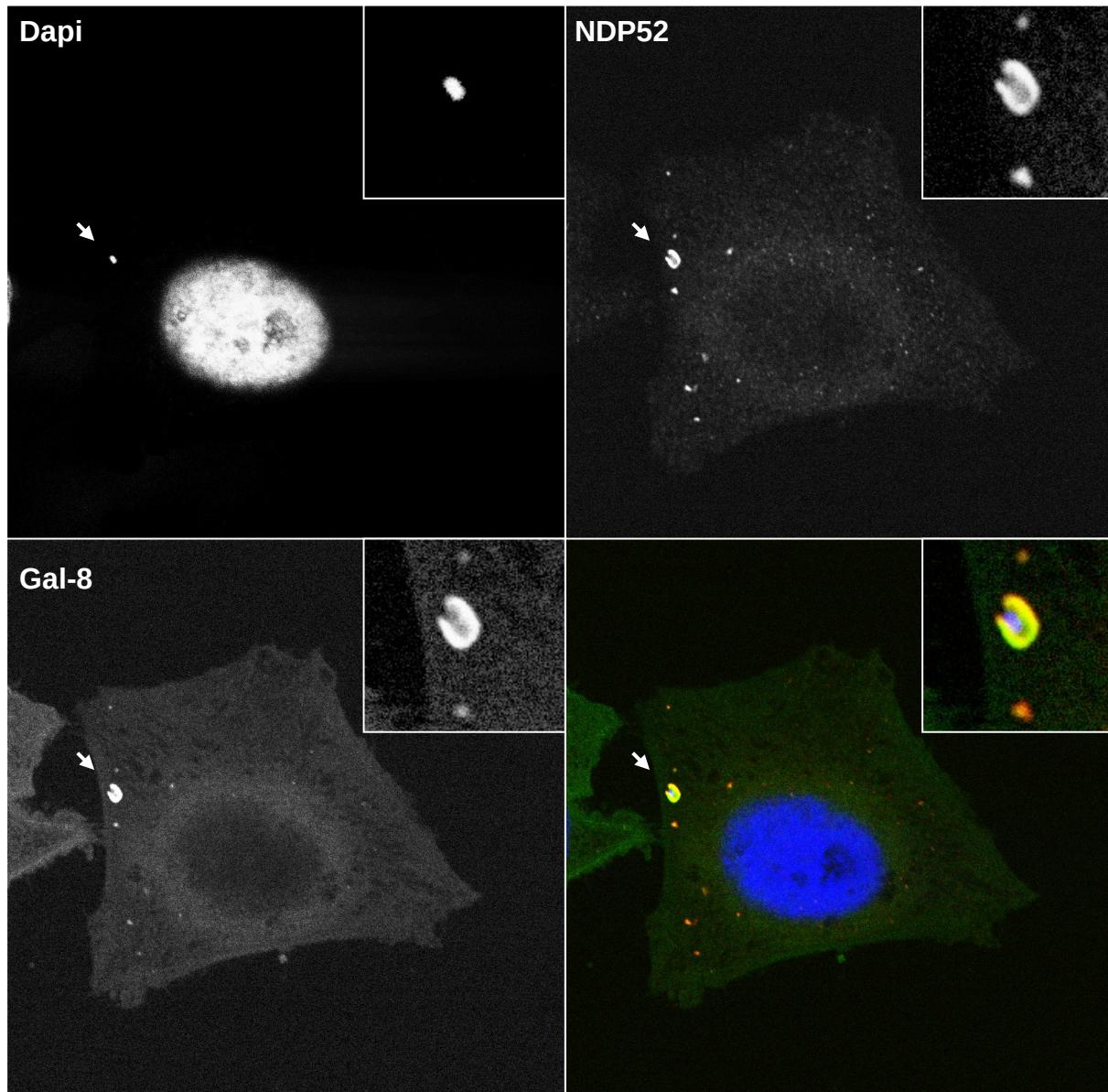
## Galectin-3, 8, and 9 detect invading Salmonella



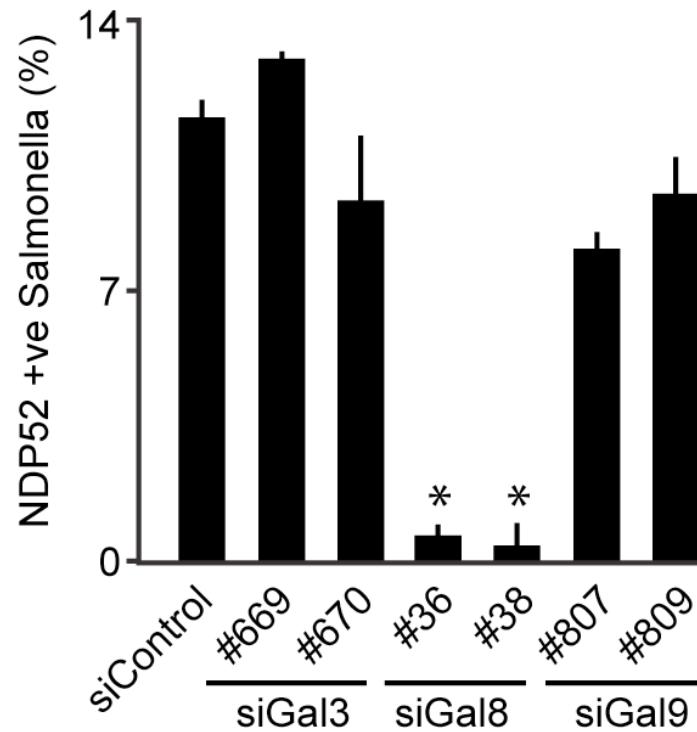
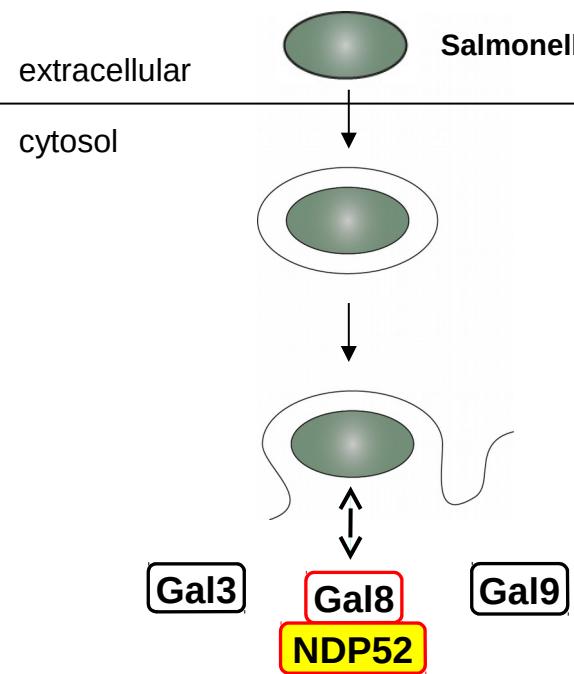
# Galectin-8 restricts the growth of Salmonella



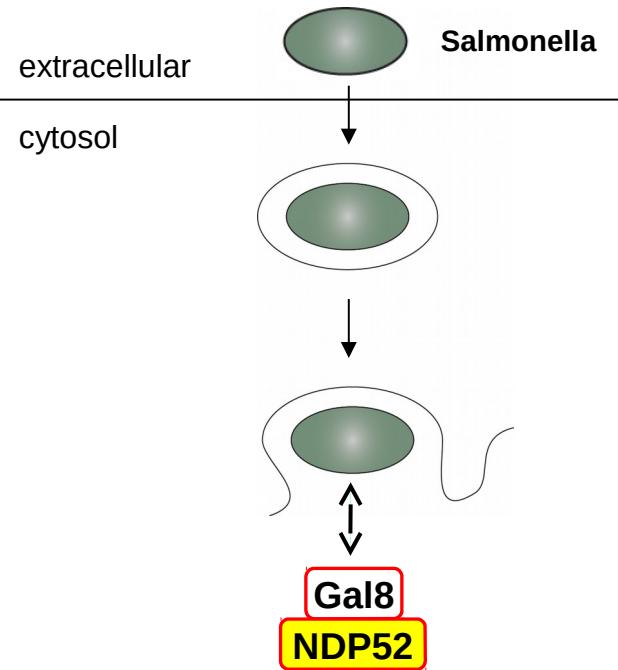
## Galectins and NDP52 co-localize on invading Salmonella



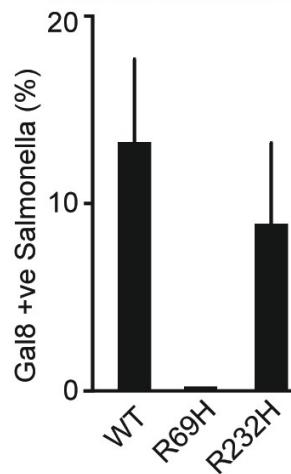
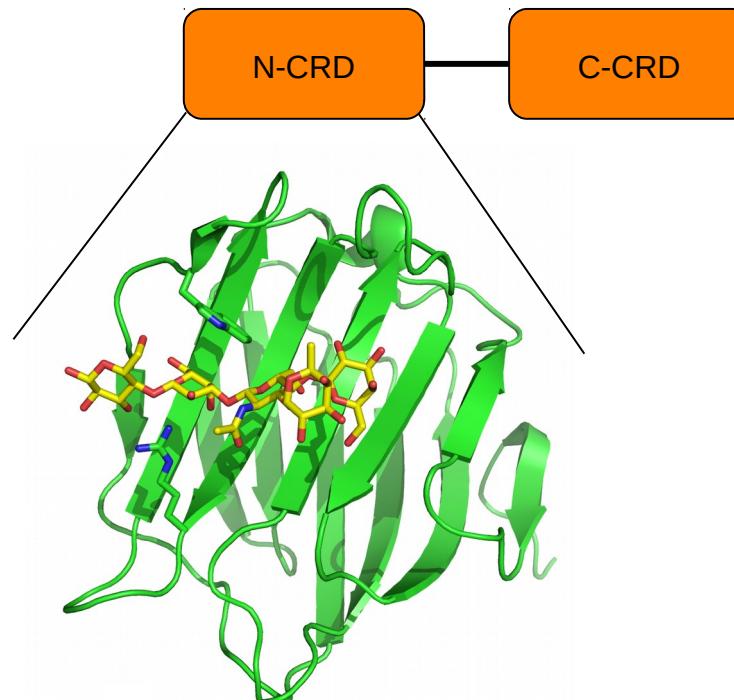
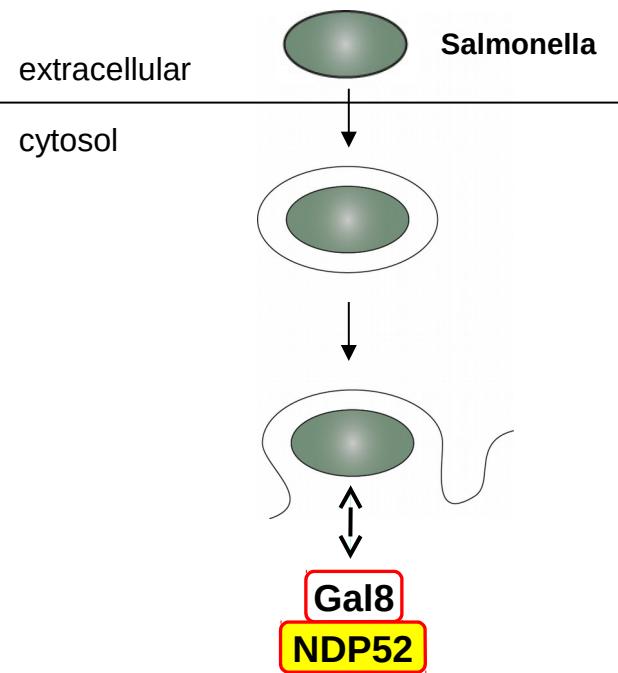
## Galectin-8 binds NDP52 and recruits it to invading Salmonella



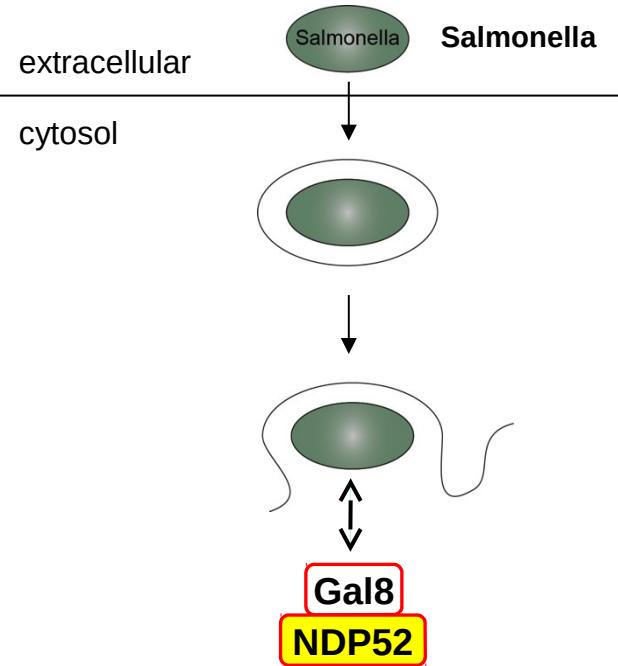
## Galectin-8 is a danger receptor that detects host glycans



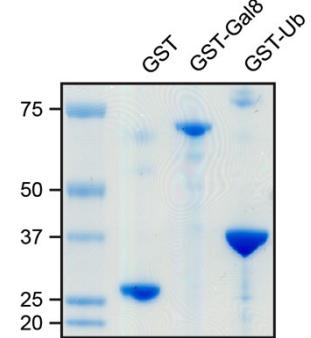
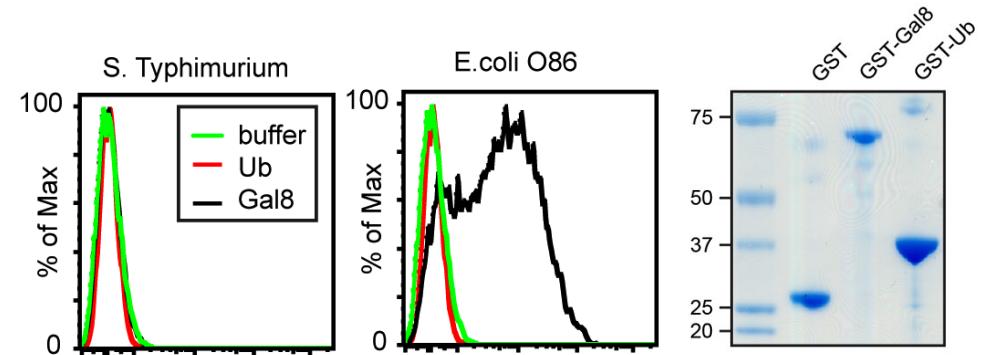
# Galectin-8 is a danger receptor that detects host glycans



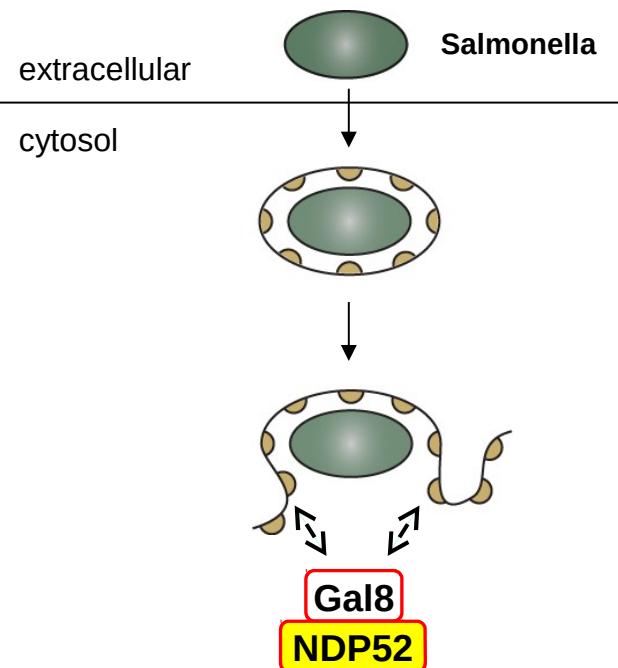
# Galectin-8 is a danger receptor that detects host glycans



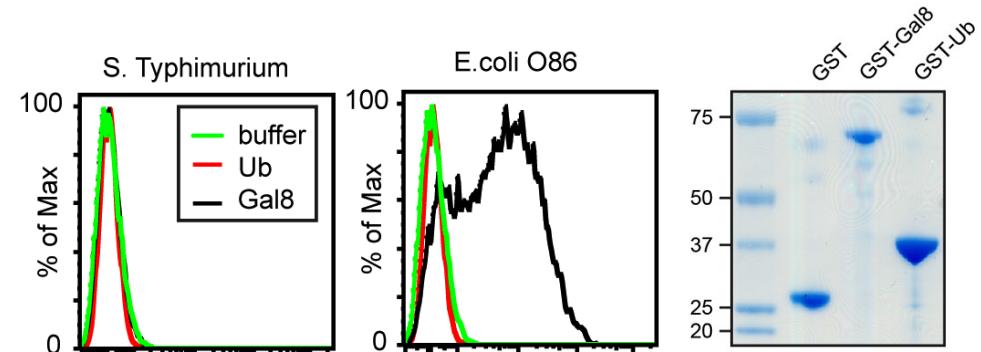
No binding of purified Galectin-8 to Salmonella *in vitro*



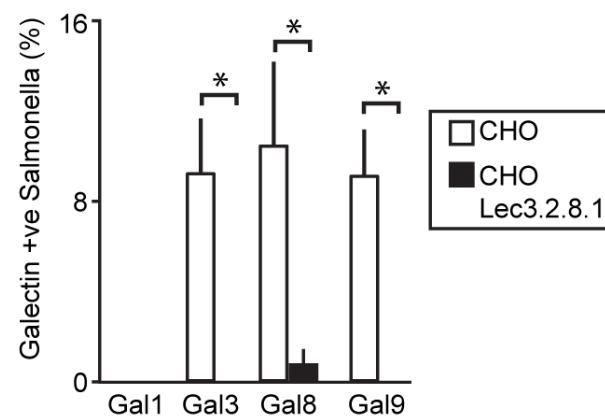
# Galectin-8 is a danger receptor that detects host glycans



No binding of purified Galectin-8 to *Salmonella* *in vitro*

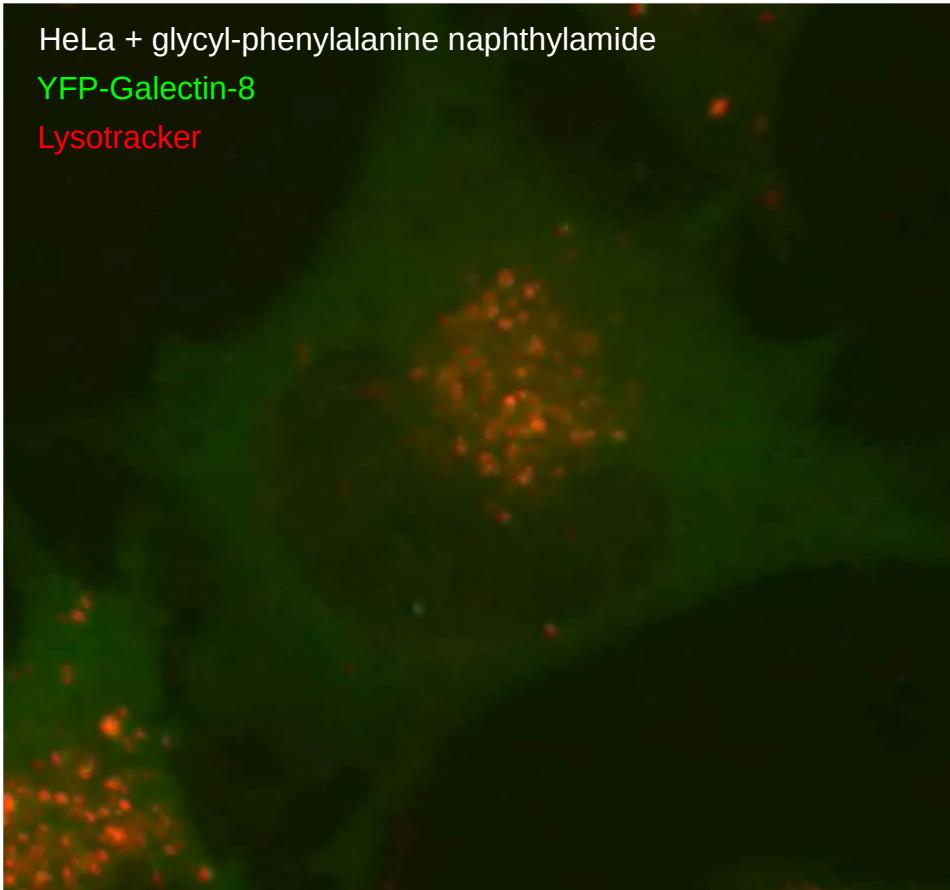


No Galectin-8 recruitment in cells lacking complex glycans



# Galectin-8 surveys the integrity of the endo-lysosomal compartment lysosomes

HeLa + glycyl-phenylalanine naphthylamide  
YFP-Galectin-8  
Lysotracker

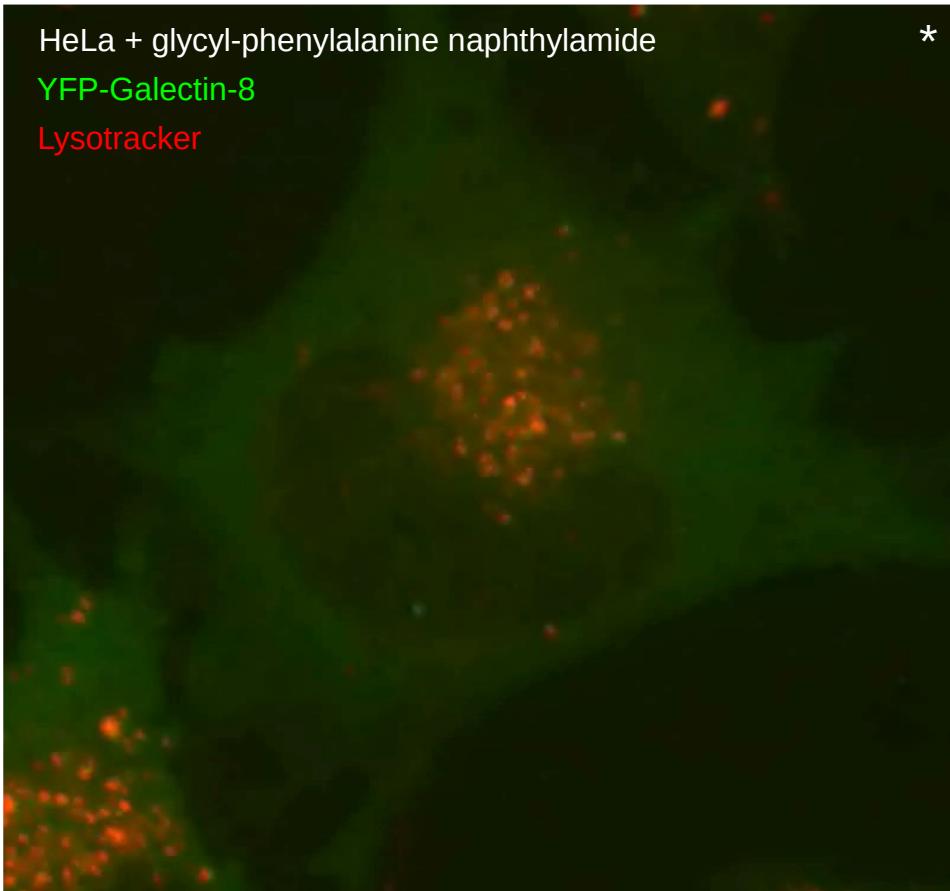


# Galectin-8 surveys the integrity of the endo-lysosomal compartment

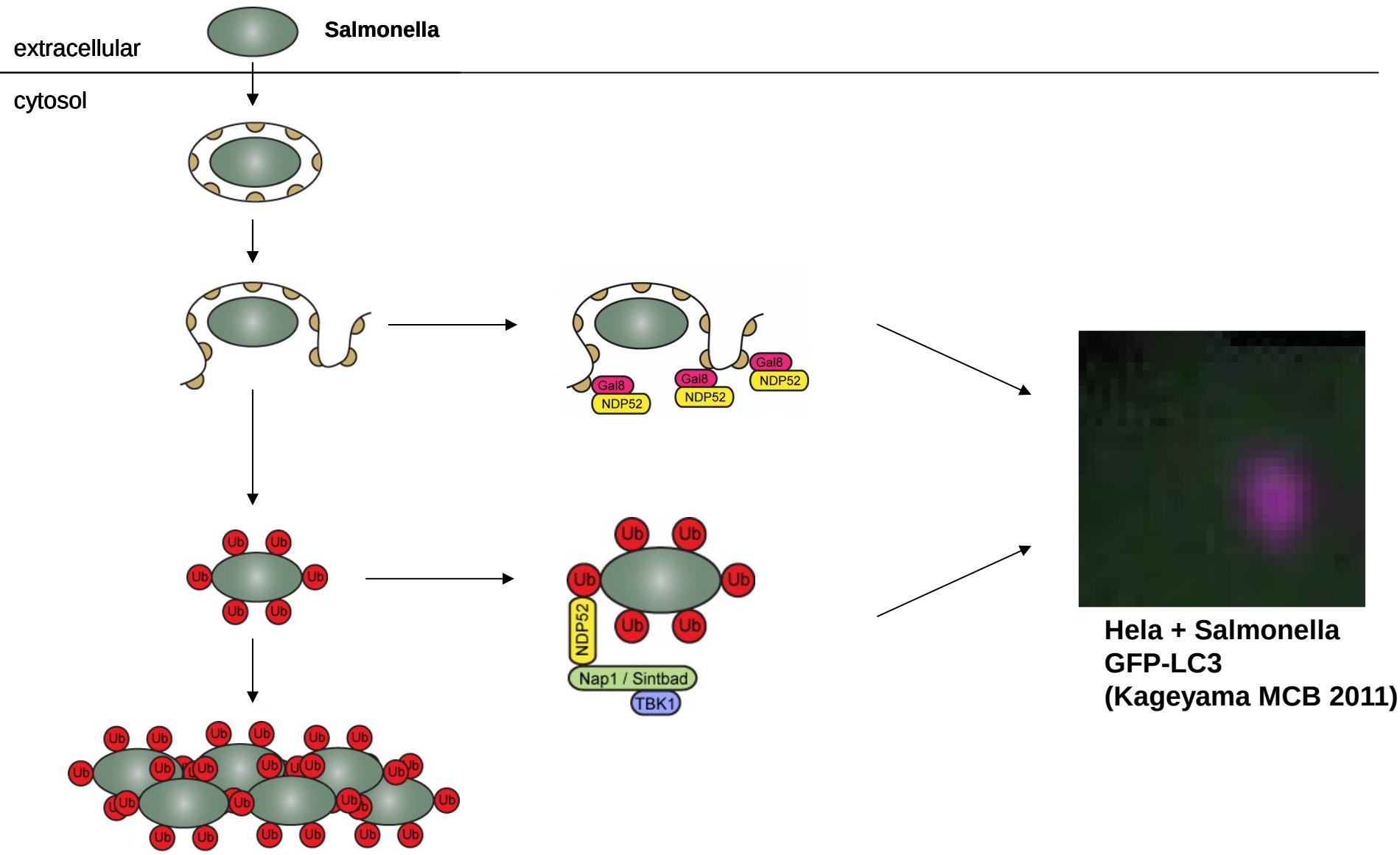
## lysosomes

HeLa + glycyl-phenylalanine naphthylamide  
YFP-Galectin-8  
Lysotracker

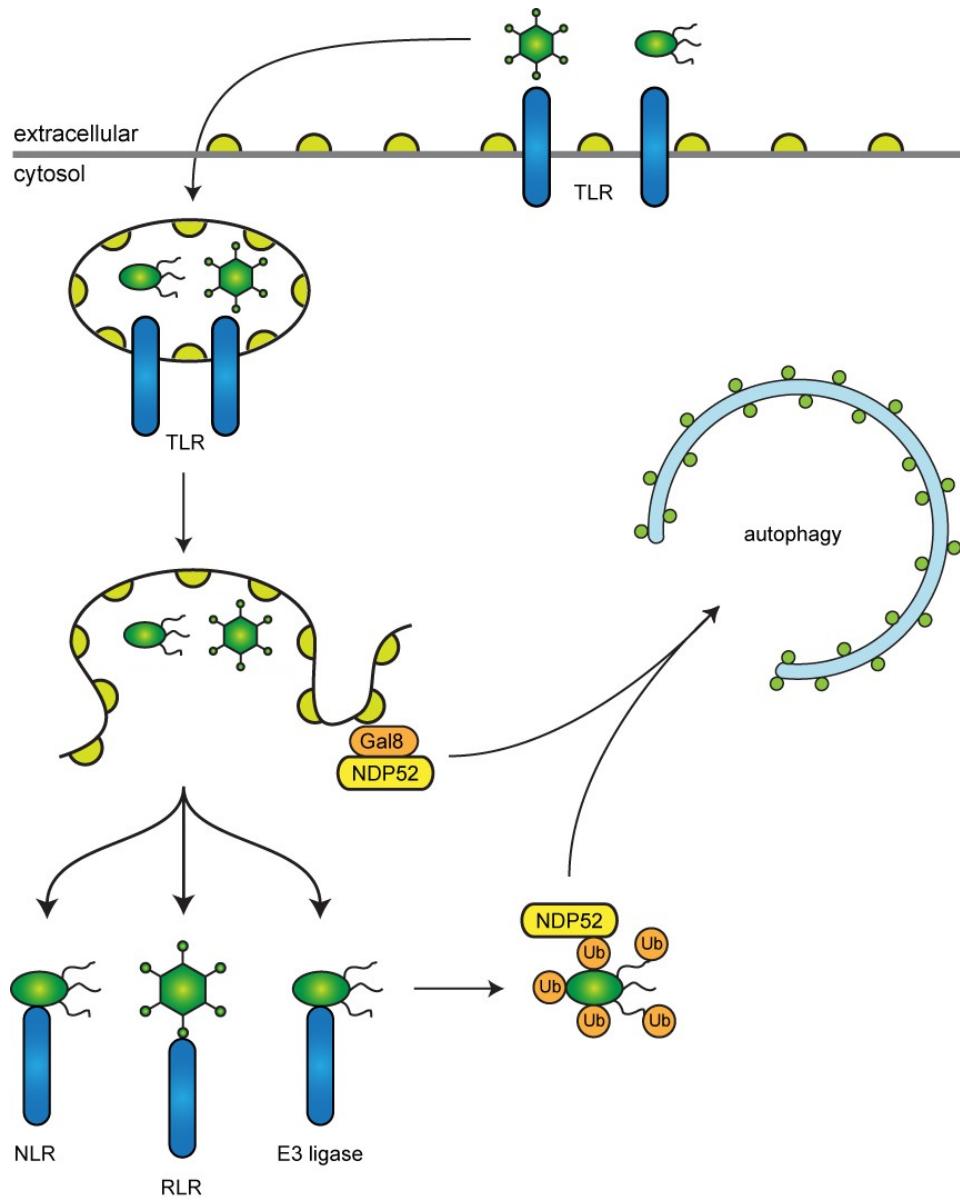
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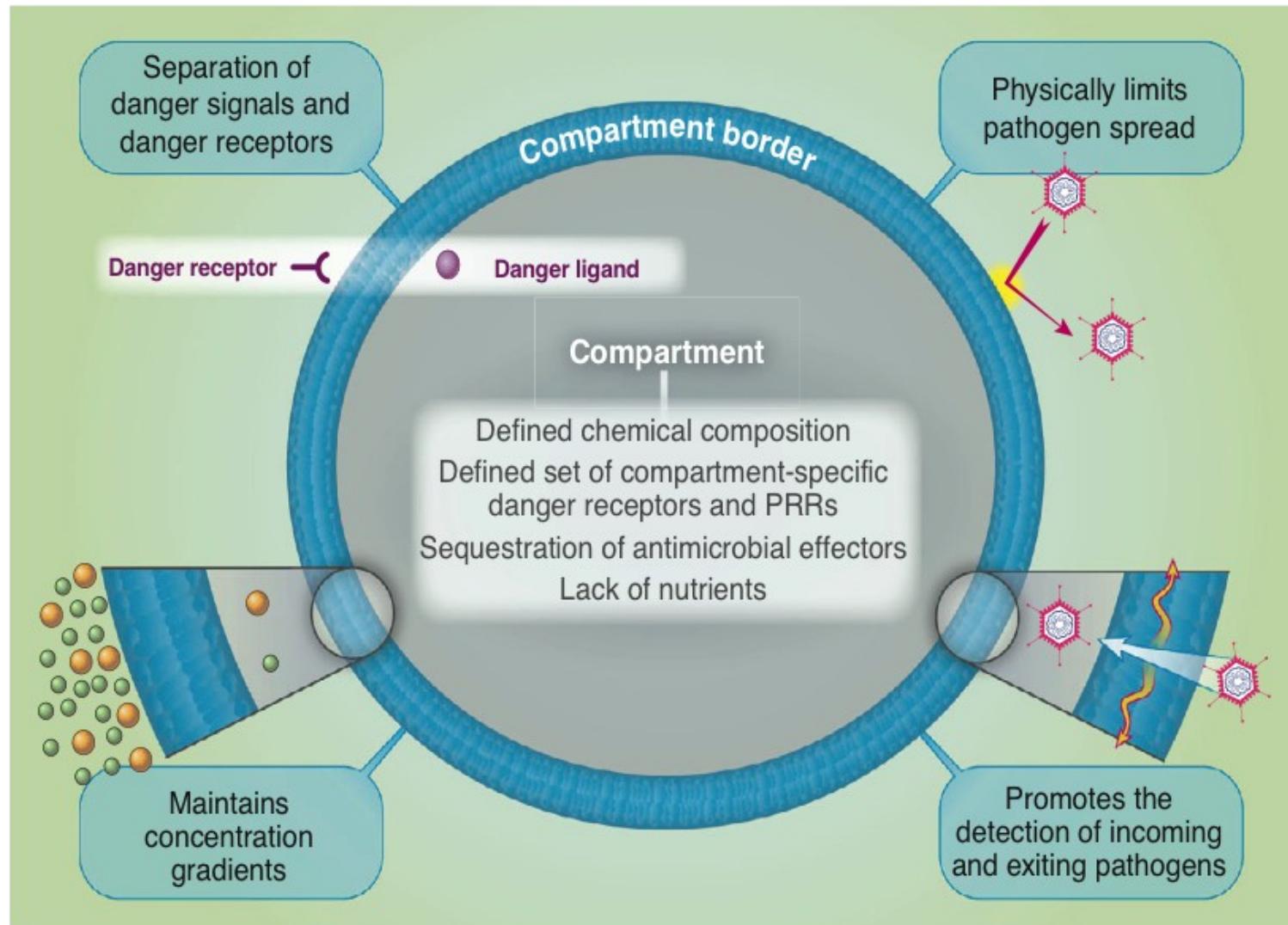
# How cells defend their cytosol against bacterial invasion



# How pattern recognition receptors and danger receptors synergize



# Compartmentalization promotes cell-autonomous immunity



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